MATERNAL-FOETAL INTERACTIONS DURING VIRAL INFECTIONS

by

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STATEMENT

The work embodied in this thesis is original and was carried out by myself except for the experiment described in Table 3.5 which was performed in collaboration with Dr. A. Mullbacher and Ms. J. Mundy.

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ABBREVIATIONS

APC	Antigen-presenting cell
ATS	Anti-thymocyte serum
B-cell	Bursa-derived lymphocyte (or equivalent)
BTVV	Bluetongue virus vaccine
CMV	Cytomegalovirus
CNS	Central nervous system
ConA	Concanavalin A
cpm	Counts per minute
CRS	Congenital rubella syndrome
fcs	Foetal calf serum
HI	Haemagglutination inhibition
^{3}HTdR	Methyl-tritiated thymidine
IBRV	Infectious bovine rhinotracheitis virus
IF	Immunofluorescence
i.p.	Intraperitoneal
i.v.	Intravenous
LCMV	Lymphocytic choriomeningitis virus
LST	Lymphocyte stimulation test
MHC	Major histocompatability complex
MLR	Mixed lymphocyte reaction
NRS	Normal rabbit serum
PALN	Para-aortic lymph nodes
p.i.	Post-infection
RRV	Ross River virus
s.c.	Subcutaneous
SI	Stimulation index
SFV	Semliki Forest virus
T-cell, T-lympho-cyte	Thymus-derived lymphocyte

ABSTRACT

The aims of the investigations presented in this thesis were to elucidate aspects of the immunology and pathogenesis of viral infections during pregnancy and to find whether cell-mediated immune responses in an infected mother have a deleterious effect on the continuation of the pregnancy. The laboratory mouse was chosen as an experimental animal because of its relative ease of breeding, storage and handling, the availability of adequate numbers of mice of different strains, and the ability to obtain pregnant mice at specified times of gestation. Model infections were established using 2 viruses, Ross River virus (RRV) and a strain of Semliki Forest virus (SFV), which are both lethal for neonatal but not adult mice.

Initial investigations examined whether the non-specific immuno-suppressive mechanisms which have been postulated to prevent maternal rejection of her foetus also depress the mother's systemic anti-viral immunity. No evidence was obtained suggesting that the state of pregnancy *per se* is associated with a systemic depression of either the stimulation or expression of anti-viral immunity in the mother. On the contrary, certain maternal T-cell mediated immune responses appeared to be markedly enhanced following infection with SFV during pregnancy. This enhanced immune reactivity was detected in the spleen, as well as the lymph nodes which drain the uterus. It was therefore suggested that the mother was hyper-immunised by a large antigenic stimulus which probably originated from the infected placental and foetal tissue within the uterus.

The effect on the pregnancy of maternal infection with either RRV or SFV was examined. Infection of 10 or 11-day pregnant mice with RRV always resulted in *in utero* death of a proportion of

the foetuses, with the others apparently unharmed. Abortion was not observed. However, infection of pregnant mice at the same stage of gestation with SFV always resulted in abortion, which was preceded by uniform foetal infection. Maternal inoculation with either RRV or SFV resulted in a haematogenous spread of virus throughout the maternal tissues, and the subsequent establishment of multiple, small infectious foci within the placental trophoblast. Foetal infection occurred at least 1 or 2 days after the initial detection of virus in the placenta. When foetal death occurred, it appeared to be a direct result of virus growth in foetal tissues.

The placenta appeared to play an important role in the pathogenesis of the in utero infections. Foetal infection was aided by the apparent predilection of both viruses for placental tissue, and their subsequent growth to high titre in that organ. Furthermore, once the placental infection had become established, both viruses persisted at high titre in the trophoblast, even though they were cleared from the mothers' tissues. It is possible that the expression of anti-viral immunity is in some way compromised in the micro-environment of the placenta, and that trophoblast may therefore be an immunologically privileged site for virus replication.

Investigations then concentrated on the reasons why some foetuses were spared from in utero infection with RRV, whereas others succumbed. The spared foetuses were found to be protected against experimental post-natal challenge with RRV, although the protection was only short-term. It was associated with the presence of anti-RRV activity in the IgG, but not IgM or IgA, fractions of their serum. These results indicated that the spared foetuses did not form an active immune response against in utero infection but were passively immunised by specific IgG derived from the

mother. It was proposed that the timing of foetal infection, relative to the timing of IgG transfer from the mother, may have been critical. When foetal infection occurred before antibody transfer, foetal death resulted, whereas when the maternal IgG was transferred to an uninfected foetus it neutralised all virus moving from the placental foci into the foetal circulation, and the foetus was spared. Anti-RRV IgG appeared to be unable to clear virus from already infected foetal tissue or the placental trophoblast.

Results obtained with SFV indicated that the outcome of this infection during pregnancy may be dependent on a similar course of events except that, following maternal infection at gestation days 10 or 11, virus invariably grew through the placenta and infected all foetuses before the transfer of protective IgG from the mother. All foetuses therefore died in utero.

The most severe effects on the pregnancy were produced by maternal infection with either RRV or SFV at gestation days 10 or 11. Infection at other stages of pregnancy resulted in a lower number of foetal deaths. It was suggested that these phenomena were linked to placental maturity rather than to any variation in foetal susceptibility throughout gestation. The less mature placenta may have been more refractory to virus growth, allowing more time for the passive protection of foetuses. Infection with SFV in early gestation also resulted in a lower incidence of abortion. It appeared that abortion was prevented when one or more foetuses within a pregnancy were spared from in utero infection. It was concluded that abortion was not triggered by virus-induced maternal ill-health, placental malfunction or any T-cell mediated immuno-pathological phenomena but was dependent upon the occurrence of uniform foetal infection. Possibly, uniform foetal infection may have resulted in an imbalance in hormone production by the foeto-placental unit which was not compatible with the normal progression of the pregnancy.

Many of the conclusions from this thesis are related to the role of maternal IgG in the pre-natal passive protection of foetuses against *in utero* viral infections. Experiments were also designed to investigate whether protection against RRV infection could be conferred post-natally, to neonatal mice. It was found that the suckling of colostrum and milk from a mother previously infected with RRV protected a susceptible neonate against homologous post-natal infection. As with the pre-natal transmission of antibody, these protected neonates only had detectable anti-RRV activity in the IgG fractions of their serum. No IgM or IgA was present, suggesting that all protective antibody was maternally derived. It was concluded that, in the mouse, all 4 IgG subclasses are selectively transferred from a mother to her offspring both pre-natally and post-natally.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1.1

REVIEW: VIRAL INFECTIONS DURING PREGNANCY AND THEIR EFFECTS ON THE DEVELOPING FOETUS

Viral infections of the mother during pregnancy are relatively common. In 1968, a survey of 30,059 human pregnancies in the United States indicated that 1600 of these women had a definite or presumed viral infection (other than the common cold) during their confinement. The actual attack rate would, in fact, be higher because no attempt was made to diagnose sub-clinical infections (Sever and White 1968). In general, the majority of these infections would be expected to have no adverse effects on foetal development. However, in some cases maternal viral infection can result in one or more of a range of syndromes in the foetus including in utero or post-natal death, abortion, congenital abnormalities, growth retardation or delayed post-natal disease (Mims 1968).

As in any other related field, the actual effect produced is the result of the interaction between the host and the infecting organism. In pregnancy, however, two hosts, the mother and the foetus, are involved, each with a susceptibility which will vary according to genetic make-up, state of nutrition, stage of gestation, and anatomical factors such as the site and structure of the placental vessels, and the site of entry of infection (Alberman and Peckham 1977).

In this Chapter it is intended to review aspects of the pathogenesis and immunology of a number of maternal infections which result in altered foetal development. Discussion will be limited to viral pathogens, however, the importance of congenital infections with bacteria, protozoa and fungi in both human medicine (Gamsu 1977) and veterinary medicine (Morgan and Wrathall 1977) should not be underestimated.

1.1.1 Congenital viral infections: extent of the problem

The first association of foetal abnormalities with a virus infection during pregnancy was proposed by

Gregg in 1941 who noticed an unusually high incidence of congenital cataract in children who had been conceived during a rubella epidemic. Most of these babies were of small size and ill-nourished, and many had congenital heart defects. Since then numerous other viruses have been shown to cause intra-uterine infections in both humans and animals (Table 1.1). In humans, the two major causes of foetal infection and damage are rubella virus and cytomegalovirus (CMV). With rubella, the natural frequency of maternal infection in the United States prior to the availability of vaccines was estimated at 8 cases per 10,000 pregnancies, rising to 220 cases per 10,000 pregnancies in epidemics (Sever and White 1968). Because of the severity of foetal lesions following maternal rubella early in gestation it is usual practice for women with laboratory confirmation of infection during the first trimester to have their pregnancies terminated. In the United Kingdom this represents 700 to 800 aborted pregnancies per year (Registrar General 1972-1973). Even so, approximately 325 children were born with congenital rubella deformities in the U.K. during 1971-1972 (Meade and Atkinson 1977).

CMV is probably the most common infectious agent to reach the foetus in humans but, unlike rubella, structural defects are rare with damage mainly being inflicted on formed organs, especially the central nervous system (CNS) (Alberman and Peckham 1977). The incidence of congenital CMV infection in New York from 1968 to 1971 was estimated to be between 60 and 150 per 10,000 live births with significant CNS damage occurring in 10% of these babies (Hanshaw 1971). In England and Wales, approximately 4000 CMV-infected babies were estimated to be born each year although, in common with the New York study, not all were expected to display overt disease (Lancet 1974). Approximately 3% of susceptible women sero-convert during pregnancy, indicating a primary infection. Of these, the risk of foetal infection is approximately 50% (Stern and Tucker 1973). However, because of the marked propensity of

Table 1.1 Viruses associated with developmental defects in animals and man[a]

Family	Virus	Species Affected
Togaviridae	rubella	human
	hog cholera (vaccine strain)	pig
	lactic dehydrogenase virus	mouse
	bovine diarrhoea-mucosal disease virus	cow
	equine infectious arteritis	horse
Bunyaviridae	Akabane	sheep, cattle
	Aino	cattle
	Rift Valley fever	sheep
	Nairobi sheep disease	sheep
Herpetoviridae	cytomegalovirus	human
		pig
		guinea pig
	varicella-zoster	human
	virus of malignant catarrh	wildebeest, cattle
	feline herpesvirus	cat
	equine rhinopneumonitis virus	horse
	infectious bovine pneumonitis virus	cattle
Poxviridae	variola, vaccinia	human
Reoviridae	reovirus types 1 and 2	mouse
	Colorado tick fever	mouse
	blue tongue (vaccine strain)	sheep
Arenaviridae	lymphocytic choriomeningitis virus	mouse
Parvoviridae	feline panleucopenia	cat
	porcine parvovirus	pig
	bovine parvovirus	cattle
	Kilhams rat virus	rat
	aleutian disease virus	mink
	minute virus	mouse
Papovaviridae	polyoma virus	mouse
	stump-tailed monkey virus	stump-tailed monkey
Retroviridae	equine infectious anaemia	horse

a. Modified from Mims (1981) and Parsonson et al. (1981).

herpesviruses, including CMV, to reactivate in an immune host, the presence of maternal serum antibody does not necessarily mean that the foetus will be protected (Stagno et al. 1973). A recent study of the incidence of congenital CMV infections in 3712 pregnant women indicated that primary and recurrent infections each resulted in 16 cases of congenital infection. However, the risk of clinically apparent disease in these children was much higher following a primary maternal infection (Stagno et al. 1982). This raises the possibility that vaccination against CMV, although fraught with difficulties, may be of value in decreasing the incidence of CMV-induced congenital defects (Medearis 1982). It is of interest that the incidence of congenital CMV and other in utero infections may be higher in lower socio-economic groups and developing countries (Mata et al. 1977; Stagno et al. 1982).

The cost to the community of human in utero infections is extremely difficult to quantify. Although the economic cost of short- and long-term social and medical care is probably not large in terms of the amount spent on public health as a whole (Meade and Atkinson 1977), the implications at the family and community levels of virus-induced foetal malformations and death may overwhelm any monetary considerations.

The problems of viral infections during pregnancy are not confined to human medicine. Infertility, reproductive failure, early embryonic death, neonatal death and abortion may result in considerable economic losses to the animal industry. The effect of these losses may be greatest in developing countries where expansion of the livestock population is frequently one of the principal aims (Ellis and Hugh-Jones 1977). Abortion rates in individual herds during epizootics may be as high as 50% with equine arteritis virus, 60% with infectious bovine rhinotracheitis virus (IBRV), and 90% with equine herpesvirus type 1 (Morgan and Wrathall 1977). In fact, IBRV is one of the major

causes of bovine abortion in certain areas of the U.S.A. (Hubbert et al. 1973). In other areas of the world, including Australia, it has recently been recognized that Akabane virus, an arthropod-borne agent, can cause extensive losses in both cattle and sheep, with over 50,000 calves estimated to have been lost in Japan between 1972 and 1980 (Bishop and Shope 1980).

1.1.2 The identification of potentially teratogenic and abortigenic viral agents

The identification of viral agents which affect foetal development may present difficulties because of the often considerable delay between maternal infection and the subsequent discovery of abnormalities in the newborn. It is further complicated when the virus causes an inapparent infection in the mother, e.g., Akabane virus (Parsonson et al. 1981). It may also be difficult to recognize that a specific syndrome exists, especially if the rate of foetal infection or abnormalities associated with a certain disease is low, or if immunity against the virus is widespread in females of reproductive age (Gershon 1975). When an infection results in early embryonic death in humans, the mother may not even realise she was pregnant (Siegel et al. 1966). Likewise, virus-induced abortion may go unnoticed in commercial herds of domestic animals (Winkler et al. 1975). Because of these problems, numerous human population surveys have been performed in an attempt to identify any cause-and-effect relationship between maternal illness during pregnancy and subsequent foetal death or abnormalities. These surveys have been either:

a) Retrospective, where women who have given birth to babies with abnormalities are questioned after the birth regarding their health status throughout the pregnancy, e.g., Gregg (1941);

or

b) Prospective, where a group of pregnant subjects are selected early in pregnancy and monitored for infections throughout confinement. A correlation is then sought between the incidence of any foetal abnormalities and the observed infections, e.g., Siegel et al. (1966).

The value of these types of surveys is, however, dependent upon a number of points. Firstly, considerable care must be taken to ensure that correct, matched controls are used, especially if the test group has been selected in any way (Siegel and Fuerst 1966; Siegel et al. 1966). Secondly, sensitive and accurate diagnostic tests should be used to confirm the nature of any infections, and reliance should not be placed on the interpretation of clinical signs (Sever and White 1968). Finally, care should also be taken in the interpretation of the results. For instance, Leck (1963), in a review of the literature suggesting that influenza caused foetal defects, doubted that there was a cause-and-effect relation because the defects observed in the offspring of women who contracted influenza during pregnancy were representative of those expected to occur in the general population and did not constitute a specific syndrome. Furthermore, malformations were unexpectedly observed in the offspring of women who contracted influenza after foetal organogenesis was complete.

It is generally agreed that prospective surveys are more accurate than retrospective surveys. The latter are likely to give a misleading estimate of the degree of risk because the abnormality being surveyed has already been selected before enquiry regarding the pregnancy. Furthermore, the mother's recollection of her health and the timing of illnesses with regard to the stage of gestation may be faulty or influenced because she has given birth to an abnormal child (Logan 1951).

Effective prospective surveys may be protracted because the frequency of viral disease is usually low in women of child-bearing age, with the result that test and control groups may be difficult to obtain (Siegel et al. 1966). Furthermore, accurate information regarding the consequences of intra-uterine viral infections may not be available until years after birth. In congenital CMV infection which is inapparent at birth, some cases of hearing loss, along with a trend towards subnormal intelligence levels, may only be observed in later childhood (Reynolds et al. 1974). Likewise, in congenital rubella syndrome, ocular, auditory and CNS anomalies may only be apparent years after birth (Jackson and Fisch 1958; Hardy et al. 1969; Menser and Forrest 1974). This necessitates thorough and long-term follow-ups of all subjects.

Thus, although a survey may provide a useful indication of the aetiologic role of certain viruses in foetal teratogenesis, the evidence is only indirect and should be backed up by serology and attempts at virus isolation (Parsonson et al. 1981). The demonstration of pre-colostral specific IgM in human babies and/or IgG in the offspring of certain domestic animals may be considered as diagnostic of intra-uterine infection because trans-placental transfer of significant amounts of the maternal immunoglobulin does not occur (Miller 1966; Stiehm et al. 1966; Morgan and Wrathall 1977). Intra-uterine infection of unknown aetiology may also be suspected in humans if the total foetal serum IgM concentration is elevated (Blattner 1974). The presence of specific foetal immunoglobulin is, however, dependent upon both the immunological maturity of the foetus at the time of infection (Silverstein et al. 1963), and, in a lethal infection, the foetus having sufficient time to produce antibody (Kendrick and Osburn 1973). Furthermore, certain viruses induce immunological tolerance in the foetus and no antibody is produced even though there may be a persistent viraemia (Plateau et al. 1980). Isolation of a virus from an affected

foetus or newborn provides excellent diagnostic evidence, however this may not always be possible because of autolysis or the presence of neutralising antibodies (Morgan and Wrathall 1977). The value of attempting virus isolations from pathological material is illustrated by a recent report of the first recorded isolation of mumps virus from a 10 week human foetus which was aborted 4 days after the onset of clinical mumps in the mother (Kurtz et al. 1982). This appears to support results obtained 16 years previously from a survey that concluded that mumps may be a significant cause of early foetal death (Siegel et al. 1966).

If virus isolation has been achieved, confirmation of its role in foetal infections is facilitated in veterinary medicine where it can be experimentally inoculated either peripherally into the mother or directly into the foetus in utero (Parsonson et al. 1981). For ethical reasons, this is not usually possible in humans although women certified for legal abortion have been infected with live rubella vaccine virus in an investigation of its safety (Vaheri et al. 1972).

1.1.3 Routes of virus entry into the foetus

Foetal infection is achieved via a number of routes (Mims 1968; Plotkin 1975; Fox 1977). The major ones are:

a) Via the germ line. The most effective route of vertical transmission is probably via integration of viral nucleic acid into the host germ line. Viruses that utilise this method must: 1) be non-cytopathic; 2) not interfere with fertilisation or embryogenesis; 3) be able to maintain infection in rapidly dividing cells; 4) avoid host immune responses (Mims 1981). Oncoviruses provide a good example of vertical transmission by this method and utilise a reverse transcriptase to integrate their genetic information into the host genome (Jarrett 1977).

When germ line cells are infected the viral genome is transmitted to embryo cells and perpetuated in all subsequent offspring. These viruses have been detected in numerous species including mice, cattle, cats, chicken and the baboon (Gross 1951; Bentvelzen et al. 1970; Kalter et al. 1973; Levy 1973; Jarrett 1977; Mims 1981), and may cause leukaemia or tumours in adult life (Gross 1951; Jarrett 1977; Mims 1981).

Infection of the germinal epithelium or unfertilised egg without integration of viral nucleic acid into the host genome may also occur, especially if there is a persistent infection of maternal tissues accompanied by immune tolerance, e.g., lymphocytic choriomeningitis virus (LCMV) in mice (Mims 1968; Plotkin 1975).

Mims (1968) and Fox (1977) also suggest that another possible, but unproven, method of vertical virus transmission via the germ line is through a provirus in the sperm nucleus.

b) "Ascending" infection. This is where the infection ascends into the amniotic sac from the vagina or cervix. This type of infection is usually peri-natal and often occurs after membrane rupture (Mims 1968; Plotkin 1975). Most cases of ascending infection are caused by bacteria but a few are fungal in origin (Fox 1977). With viruses, ascending infection may be important in peri-natal intra-uterine infections with herpes simplex virus (HSV) (Nahmias et al. 1971; 1975), and has also been postulated to be responsible for a proportion of foetal infections with rubella (Seppala and Vaheri 1974).

As noted previously in Section 1.1.1, latent herpesvirus infections are commonly subject to recrudescences (Weller 1971). Activation of a latent CMV infection, with accompanying shedding

of virus from the cervix, often occurs during pregnancy (Montgomery et al. 1972). It is therefore possible that when intra-uterine infection occurs following CMV reactivation in women who have pre-existing serum antibody (Stagno et al. 1982), the route of infection is probably ascending rather than haematogenous (Lowrie et al. 1977).

Ascending infections may also occur when viruses are present in semen (Mims 1981). It is possible that this occurs in border disease in sheep where the virus has been transmitted by semen from infected rams to susceptible ewes (Gardiner and Barlow 1981). Furthermore, in humans, CMV may be persistently shed in semen for up to 14 months after infection, suggesting the possibility that some intra-uterine infections by this virus may be sexually transmitted (Lang et al. 1974; Embil et al. 1982).

c) Via the chorioallantoic placenta. The chorioallantoic placenta is found in all eutherian mammals although its structure and the number of cell layers separating maternal and foetal circulations depend upon both the species of animal and the stage of gestation (Enders 1965; Mims 1968). In both humans and rodents this placenta is of the haemochorial type where foetally-derived trophoblast tissue is in intimate contact with the maternal blood (Enders 1965; Pijnenborg et al. 1981). Viruses which cause in utero infections often have a predilection for the chorioallantoic placenta which becomes infected after a haematogenous spread of virus from the site of infection in the mother (Fox 1977). The release of infectious progeny virus from the placental focus of infection then results in foetal infection. A direct passage of virus in maternal leukocytes or erythrocytes across the placenta may also occur (Catalano and Sever 1971; Lowrie et al. 1977),

although there is little direct evidence that this constitutes an important mechanism of foetal infection. The possibility of a direct leakage of virus from the maternal to the foetal circulation must also be considered because of the results of Uhr et al. (1963) who found that bacteriophage crossed the guinea-pig placenta when high concentrations (10^7 phage/ml) were present in maternal serum.

Other possible routes of virus entry into the foetus are via the endometrium, yolk sac, fallopian tube, and surgical procedures such as amniocentesis or intra-uterine transfusions (Mims 1968; Plotkin 1975; Fox 1977). However, infection via these routes rarely, if ever, occurs.

1.1.4 The role of the placenta as a barrier to infection.

Although the placenta is often considered as a total barrier to the free passage of organisms from the maternal circulation to the foetus, Fox (1977) suggested that at best it can only physically delay foetal infection. Nevertheless, virus has been isolated from placental tissue in the absence of foetal infection in human rubella (Alford et al. 1964) and CMV infections (Hayes and Gibas 1971), as well as in animal infections with LCMV (Mims 1968),CMV (Johnson 1969) and IBRV (Kendrick et al. 1971; Kendrick 1973). It is possible that in these examples the foetus is resistant to infection, although this can be tested, at least in animals, by direct in utero inoculation of the agent.

Placental infection without foetal infection does not necessarily spare the foetus. Foetal death may occur as a direct consequence of virus-induced changes in the placenta and placental circulation involving vasoconstriction, congestion or haemmorrhage

(Mims 1976b). This may be rare, however, because, although a number of viruses do cause focal villous inflammation in humans, the placenta has a large functional reserve capacity and is unlikely to be compromised in its efficiency (Fox 1977; 1981). Infection of mice with murine CMV is an example where foetal death without infection is common and appears to be associated with virus replication in the placenta (Johnson 1969). However, the possibility that infection causes inapparent changes in the mother which are incompatible with foetal life must also be considered.

1.1.5 The expression of anti-viral immune responses in the placenta.

Johnson (1969) speculated that intrinsic placental antibody formation as well as interferon production by the trophoblast may be responsible for the failure of murine CMV to cross from the placenta to the foetus. It seems likely, however, that specific placental anti-viral immune mechanisms such as antibody production, if present, would only develop at the same rate as in the foetus and this, in turn, depends upon the species in question. In the mouse, the developing foetus appears to be immunologically incompetent (Silverstein 1972), and the full functional expression of antibody and cell-mediated immune responses may not be gained until after birth (Makinodan and Peterson 1962; Dalmasso et al. 1963; Chiscon and Golub 1972). On the other hand, interferon may be important in limiting trans-placental virus spread (Fox 1977). Interferon is produced by foetal and placental tissue in a number of species (Isaacs and Baron 1960; Banatvala et al. 1971), and progressively concentrates in the mouse placenta throughout pregnancy (Fowler et al. 1980). It has been suggested that the reason why Japanese strains of rubella do not appear to cross the human placenta as often as other strains (Kono et al. 1969) is that the Japanese strains are much better inducers of interferon

in placental tissue (Banatvala et al. 1971; Potter et al. 1973). An alternative explanation, however, is that the Japanese race lacks certain HLA antigens which may determine susceptibility to rubella infection (Honeyman et al. 1975).

In certain species, the mother may also contribute to the level of anti-viral immunity in the foeto-placental unit by the passive transfer of certain antibody classes (Miller 1966; Stiehm et al. 1966; Morgan and Wrathall 1977; see also Chapter 1.3). However, it seems likely that the expression of maternal cell-mediated immune responses may be restricted or inhibited in the placenta. Numerous mechanisms have been postulated to explain why an allogeneic foetus is not rejected as foreign by the mother's immune system (reviewed in Chapter 1.2), and it is possible that viruses able to infect the placenta may utilise these same mechanisms to evade the maternal immune response and set up a persistent placental infection. This may be expected to facilitate foetal infection. Thus, in some respects, the placenta may be an immunologically "privileged" site for virus replication.

1.1.6 Sequelae of virus infections in pregnancy

1.1.6.1 Congenital abnormalities

Virtually all congenital malformations of infective origin are caused by viruses because other organisms such as bacteria tend to release toxins resulting in extensive tissue damage with subsequent foetal death (Mims 1976b). During the embryonic period, which is characterised by cellular differentiation and early organ formation, exposure to teratogens is more likely to produce structural abnormalities, whereas exposure during the foetal period, which is characterised by growth and functional maturation, is more likely to cause disturbances in these developmental processes (Morgan and Wrathall 1977). This is exemplified in

human rubella where the risk of congenital malformation is dependent upon the stage of pregnancy at which infection occurs. Maternal infection during the first trimester represents the greatest risk to the foetus (Dudgeon 1969), with malformations of the heart, ear, eye and brain common (Banatvala 1977). However, in utero infection with rubella can occur at any stage of gestation (Cradock-Watson et al. 1980). Children from mothers infected during the second trimester may have more subtle disease manifestations such as impaired hearing and mental development which are not apparent at birth (Hardy et al. 1969). This is considered to be a consequence of the establishment of a chronic infection rather than a direct teratogenic effect on organ development. In vitro studies on the mechanisms of the rubella-induced teratogenic effects indicate that the virus causes chromosomal damage, a decreased mitotic rate and a reduced cell life (Plotkin et al. 1965; Rawls and Melnick 1966; Hoskins and Plotkin 1967; Plotkin and Vaheri 1967). Thus, the low birthweight often observed in infants with congenital rubella may be due to a decreased number of body cells and altered RNA and protein synthesis (Naeye and Blanc 1965; Hill et al. 1970). Virus replication in foetal endothelial cells can result in areas of necrosis in affected tissues which may also contribute to the disease pathogenesis (Tondury and Smith 1966).

A variation in the foetal susceptibility to virus damage at different stages of gestation is not unique to rubella. In other diseases, the specific target organs or cells may only be present or susceptible during a brief period of pregnancy. For example, when live bluetongue virus vaccine (BTVV) is given to pregnant ewes between gestation days 50 to 58 the foetuses develop a severe necrotising encephalopathy which presents at birth as hydranencephaly. Infection at 70 to 78 days of gestation results in a multifocal encephalitis and vacuolation of white matter which presents at birth as porencephalic cysts. However, if the vaccine

is given after 100 days of gestation only a mild focal encephalitis with no pathologic sequelae except glial nodules is observed (Osburn et al. 1971a). Two factors were thought to account for this marked variation in susceptibility of the foetal sheep to BTVV:

a) The predilection of the virus for nervous tissue in the young foetal lamb may be due to a selective vulnerability of undifferentiated neural cells to the infection. Increased resistance of foetal cells to infection appears to be associated with tissue differentiation and maturation.

b) The late maturation of immune competence in the foetal lamb (specific serum-neutralising antibodies against BTVV may not be induced until after 122 days of gestation) allows infection prior to this time to proceed unimpaired (Osburn et al. 1971b).

A similar situation may be involved in the pathogenesis of Akabane virus infection of foetal sheep where abnormalities are only observed when pregnant ewes are infected between 30 and 36 days of gestation, even though there is serological evidence of foetal infection after maternal inoculation at other stages of pregnancy (Parsonson et al. 1977).

The deleterious effects of virus infections are not always limited to early gestation foetuses. Pathology associated with feline panleukopenia virus is most severe when foetuses are infected late in gestation, with the greatest destruction occurring in the cerebellum which undergoes growth activity at this time and in the neonatal period (Kilham et al. 1967). Furthermore, some viruses may persist in foetal cells in latent form doing little or no damage until the susceptible period of development occurs when their teratogenic effect will be expressed. This has been suggested to occur with certain strains of swine fever virus

in pigs (Morgan and Wrathall 1977).

1.1.6.2 Foetal death

Foetal death tends to result from infections with highly cytolytic viruses that cross the placenta. When infection occurs in early gestation the dead embryo may be resorbed whereas later in gestation, after skeletal calcification has occurred, resorption may be incomplete with resultant mummification and abortion (Taylor-Robinson 1977). In polytocous species, abortion does not usually occur unless all foetuses die (Arthur 1975), presumably because only one viable foetus is required to maintain the hormonal balance necessary for the continuation of the pregnancy. Numerous viruses have been demonstrated or are suspected to cause foetal death. In humans these include: rubella, where foetal death occurs in approximately 50% of all cases when the mother contracts the disease in the first 8 weeks of gestation, and in approximately 20% of all cases during the third month (Siegel and Greenberg 1960); mumps, which is associated with a 32% foetal death rate if contracted during the first trimester (Siegel et al. 1966); HSV, which has a high abortion rate associated with primary genital lesions during the first 20 weeks of gestation (Nahmias et al. 1971; Florman et al. 1973); and smallpox which, although not a health problem today, is included because it has caused an extremely high rate of foetal death and abortion in previous epidemics. In an Indian study, 72% of women who contracted small-pox in the first 25 weeks of gestation had foetal loss. This decreased to a 39% loss when the disease was contracted during the 25th to 36th week of gestation. There was a 10% incidence of stillbirth in those cases admitted at full term (Rao 1972).

Some viruses known to cause a significant amount of foetal wastage in animals are: IBRV which, in cattle, frequently causes abortion (Kendrick and Straub 1967); swine fever virus which causes an acute disease

in pigs and, depending upon the virulence of the infecting strain, can result in abortion and malformations (Morgan and Wrathall 1977); Akabane virus which, as previously noted, caused the loss of over 50,000 calves in Japan between 1972 and 1980 (Bishop and Shope 1980); bovine viral diarrhoea - mucosal disease virus which causes foetal death in both cattle (Done et al. 1980) and sheep (Parsonson et al. 1979). It has been suggested that the latter virus may be the most important pathogen of the bovine foetus in Great Britain (Done et al. 1980).

Although the usual cause of foetal death is viral replication within foetal tissues, it may result from a disturbance in placental function. Viral infections may also cause a variety of disturbances in maternal health including hyperthermia, hypoxia, biochemical imbalance or a nutritional disorder, any one of which may result in a detrimental effect on the foetus (Alberman and Peckham 1977). An elegant example of the influence of maternal health on foetal well-being is provided by the infection of pregnant mice with coxsackievirus B3 which leads to severe pancreatic acinar atrophy and hepatitis in the mother, and death or retarded development in the foetuses. It was suggested that the infected mothers may have had a dietary insufficiency attributable to an inability to break down and digest protein in their diet (Lansdown and Coid 1974), and this was supported when a dietary supplement of casein hydrolysate including free amino acids and simple peptides corrected the condition and allowed the pregnancy to proceed normally (Lansdown 1975).

1.1.6.3 Delayed post-natal disease in the progeny

In section 1.1.3 it was noted that oncoviruses are transmitted vertically by the germ line and may cause tumours or leukaemia in adult life. Association between infection in pregnancy and subsequent malignant disease has also been suggested for a number of other viruses including influenza, varicella and possibly

rubella (Fedrick and Alberman 1972; Blithel et al. 1973). Conversely, Leck and Steward (1972) found no such association with influenza. Although there is overwhelming evidence that oncoviruses are able to initiate delayed neoplastic disease in the offspring, the evidence for the other viruses is not as strong, mainly because the surveys on which the statistical evaluations were performed may have been inadequate in a number of respects (Leck 1963; Blot et al. 1980).

Other, non-malignant, examples of delayed post-natal disease in the progeny of mothers infected during pregnancy include reovirus type 2 infection in mice, where decreased activity and growth retardation occur in some progeny between 15 and 36 days of life (Hashimi et al. 1966), and human rubella, where a chronic, progressive panencephalitis may be an infrequent complication in infants with congenital rubella syndrome (Weil et al. 1975).

1.1.6.4 Virus persistence in the progeny

Burnet and Fenner (1949) postulated that at some stage of embryogenesis the foetus develops a recognition mechanism when all antigens present are acknowledged as self antigens and are unable to thereafter stimulate an immune response in that host. In accord with this, viruses which infect a foetus at an early stage of development and do not prejudice its pre- or post-natal survival should also be recognized as self. This may lead to the establishment of a persistent infection, although the level of this persistence may be influenced by maternal antibodies acquired trans-placentally or via colostrum. There are, in fact, numerous reports of virus persistence for weeks, months or even years following in utero infections with such agents as swine fever in pigs (Huck and Aston 1964), human rubella (Banatvala et al. 1965); reovirus type 2 in mice (Hashimi et al. 1966), feline panleukopenia virus (Kilham et al. 1967), LCMV in mice (Skinner and

Knight 1971), human CMV (Stagno et al. 1975), aleutian disease virus in mink (Porter et al. 1977), bluetongue virus in sheep (Gibbs et al. 1979), border disease in sheep (Barlow et al. 1980), hog cholera in pigs (Plateau et al. 1980) and bluetongue virus in North American elk (Stott et al. 1982). Such persistence may aid the spread of disease to other susceptible humans or animals (Huck and Aston 1964; Banatvala et al. 1965; Skinner and Knight 1971; Plateau et al. 1980), and may allow "overwintering" of arthropod-borne diseases (Gibbs et al. 1979; Stott et al. 1982). There seems to be two mechanisms involved in this virus persistence. Firstly, immunological tolerance, as assessed by the lack of a specific antibody response of the host to the virus, and as predicted by Burnet and Fenner (1949), may occur (Hashimi et al. 1966; Skinner and Knight 1971; Porter et al. 1977; Barlow et al. 1980; Plateau et al. 1980; Stott et al. 1982). Secondly, although the foetus may recognize the viral antigen as foreign, its immune response may be defective in some way and unable to clear the infection. For example, almost all children severely affected with congenital rubella syndrome excrete virus at birth and some may continue to excrete virus for months thereafter (Banatvala et al. 1965). This is not due to immunological tolerance because neutralising IgM antibodies are present (Alford 1965). However, when the immune responses of these children are more closely examined, defects in both humoral and cell-mediated anti-viral immunity may be observed (Soothill et al. 1966; Fuccillo et al. 1974). Whether these defects in the affected child's immune response precipitate virus persistence or occur as a consequence of it is not clear.

1.1.7 Immuno-therapy against congenital virus diseases

1.1.7.1 Prophylactic immuno-therapy

A good example of the successful use of immuno-prophylactic measures to decrease the incidence of virus-induced congenital abnormalities is provided by the human rubella vaccination programme. Tobin et al. (1977) noted that prevention of the birth of rubella damaged children can be accomplished in three ways:

1) By identifying those pregnancies at risk and terminating them.

2) By inducing immunity with live vaccine in women before pregnancy.

3) By mass vaccination of school children to reduce the level of natural infection circulating in the community.

Obviously, vaccination programmes are more socially acceptable than the first alternative! In the United States a live rubella vaccine became available in 1969, and in the first 5 years of use $5.5X10^7$ doses were dispensed (Krugman and Katz 1974). Initial control measures were aimed at pre-school and young school-age children in order to decrease the overall number of rubella cases in the community. Vaccination of susceptible post-pubertal females was given a lower priority. Although this did result in a significant decrease in the incidence of rubella in the total population, and an initial decrease in the incidence of congenital rubella syndrome (CRS) (Krugman and Katz 1974), the overall incidence of CRS has not declined in recent years (Orenstein and Greaves 1982). A change in vaccination strategy was therefore made in the mid-to-late 1970s with greater emphasis being placed on vaccination of susceptible females of childbearing age.

Orenstein and Greaves (1982) suggested that this has been only partly successful, however, because of:

a) inadequate education of the general population;

b) insufficient input by physicians and health workers into education and vaccination of susceptible people.

Furthermore, they considered that possibly the only way to further decrease the incidence of CRS was to make vaccination compulsory for all students from kindergarten to 12th grade, as well as in all other institutions where young adults congregate.

Although the vaccine strains in current usage (HPV77, Cendehill and RA27/3) are effective, they may not induce as high a level of immunity as the wild-type virus. There are both qualitative and quantitative differences in the antibody responses to rubella vaccination compared to the natural disease (Ogra et al. 1971; Al-Nakib et al. 1975). Furthermore, natural reinfection may be more common in vaccinated people because of lower antibody levels (Lancet 1973). A recent study also indicated that vaccine-induced immunity may not be as long lasting as that induced by natural disease. Over 90% of 13 to 14 year old school girls had detectable anti-rubella antibodies 7 years after vaccination with RA27/3 or Cendehill, although 13 to 14% had only low titres. After natural infection, however, 98.1% of girls were immune and only 4.5% had low antibody titres (Zealley and Edmond 1982). This study also illustrated that a proportion of both vaccinated and naturally infected people have no detectable antibody to rubella. Whether this is because sero-conversion never occurred (Menser and Forrest 1975) or antibody has just disappeared is not clear.

A major problem with the use of live rubella vaccines is the possibility of accidental vaccination during

pregnancy, especially in early pregnancy when the mother may not be aware of her condition. This has been raised as an objection to the widespread and indiscriminate vaccination of all post-pubertal females (Orenstein and Greaves 1982). There are reports of the isolation of virus from the placenta and occasionally the foetus after therapeutic abortion of initially sero-negative pregnant women who were vaccinated in early pregnancy (Larson et al. 1971; Ebbin et al. 1972; Vaheri et al. 1972; Modlin et al. 1976), although the incidence of foetal infection appears to be much lower than would be expected with wild-type virus. It is not clear whether infants infected in utero with the vaccine virus will show any post-natal damage. Modlin et al. (1976) found no defects in 38 infants born to susceptible mothers after vaccination in early pregnancy. Likewise, Preblud et al. (1978) found no defects in the offspring of 65 susceptible women vaccinated during pregnancy, even though 2 infants had laboratory evidence of congenital infection. It is, however, essential that these infants are followed over a number of years in case any more subtle problems, such as partial deafness, appear. Possibly in the light of these results the Immunization Practises Advisory Committee, U.S.A. (1981) suggested that rubella vaccination during pregnancy should not be a reason to routinely recommend therapeutic abortion.

1.1.7.2 Therapeutic immuno-therapy

Where active immunity against infection does not exist in, for example, a susceptible pregnant woman exposed to rubella, passive immunisation with anti-rubella immunoglobulin has been attempted. Although Peckham (1974) indicated that this may be partially effective in protecting the foetus against rubella infection, these results are not supported by other workers (Butler et al. 1965; Forrest and Menser 1974). In fact, there appear to be many problems which mitigate against the widespread usage of this treatment: high titred serum is not routinely available; the infectious

contact may not be apparent if the disease was sub-clinical; it is extremely important that treatment is commenced as soon as possible after contact; the patient may not be aware that she is pregnant at the time of contact.

CHAPTER 1.2

REVIEW: THE EFFECT OF PREGNANCY ON THE EXPRESSION OF ANTI-VIRAL IMMUNE RESPONSES

Pregnancy is the only naturally occurring example of successful allografting. The foetus, in an outbred mating, expresses paternal Major Histocompatability Complex (MHC) antigens but is not rejected by the mother. The immunological paradox of pregnancy is perhaps best stated by Faulk and McIntyre (1981) who noted that, in evolutionary terms, the more primitive animals had already developed a complex system of cellular recognition and rejection mechanisms before the appearance of eutherians. Thus, if mechanisms for the rejection of non-self antigens existed before the advent of placentation, how did a system of reproduction develop that depended for its survival upon the co-existence of allogeneic tissues, and the live birth of young rather than the laying and hatching of eggs?

Available evidence suggests that the mother does become immunologically primed against foetal MHC antigens after 1 or more pregnancies (Kaliss and Dagg 1964; Hellstrom et al. 1969; Maroni and deSousa 1973; Youtananukorn et al. 1974; Rocklin et al. 1976; Chaouat et al. 1979; Bell and Billington 1980; 1981). It has also been established that immunisation of the mother against paternal antigens either before or after conception does not affect the course of the pregnancy (Medawar and Sparrow 1956; Beer and Billingham 1979). It appears, therefore, that the expression of immune reactivity against the foetal tissues exposed to the mother is either ineffective, suppressed or blocked.

The mechanisms involved in ensuring that the allogeneic foetus is not rejected have not yet been elucidated although many hypotheses have been put forward. The aim of this Chapter is to review some of these proposals, in particular those which would be expected to have a more general influence on the maternal immune response against viral infection.

1.2.1 Is the gravid uterus an immunologically privileged site?

It is generally considered that the gravid uterus is not endowed with distinctive characteristics that make it a favourable site for the implantation of allogeneic tissue (Billingham 1964; Beer and Billingham 1974; Stites et al. 1979). In fact, the occasional occurrence of extra-uterine pregnancies suggests that the mechanisms involved with the maternal inertia against the foetus are probably governed by the foeto-placental unit (Siiteri and Stites 1982). Nevertheless, there is evidence that the decidual tissue situated beneath the developing rat zygote may interfere with the drainage of embryonic antigen into the maternal lymphatics and delay the development of maternal reactivity against the foreign antigens (Beer and Billingham 1971; Beer et al. 1971). Although this is not considered to play a significant role in ensuring the survival of the embryo, it may act to delay the development of the maternal immune response against agents causing intra-uterine infection.

1.2.2 Trophoblast antigenicity

In all mammalian species trophoblast is the only foetal element in direct and continuous contact with maternal tissues in the placenta. In man, other primates and rodents, the chorioallantoic placenta is of the haemochorial type where the uterine capillaries are breached and the trophoblast is bathed in maternal blood (Billington 1975). As the placenta is foetal in origin, its close juxtaposition to the maternal immune response necessitates the existence of mechanisms which render the trophoblast insusceptible to rejection. These mechanisms may also be relevant in governing the expression of anti-viral immunity in the placenta.

Beer and Billingham (1978) considered that the most likely explanation for the non-rejection of all-

ogeneic foetuses by their mothers is that the trophoblast is an immunologically privileged tissue. They cited 4 reasons for this:

1) Ectopic pregnancies in various parts of the body are usually successful and their demise, if it occurs, is not immunologically mediated.

2) Choriocarcinoma, a trophoblastic neoplasm of embryonic origin, is completely refractory to transplantation immunity directed against paternal antigens.

3) Trophoblast (trophectoderm) develops very early, before implantation.

4) Small grafts of trophoblastic tissue obtained from ectoplacental cones appear to be invulnerable to transplantation rejection.

These peculiar properties of trophoblast were originally attributed to an electron-dense acidic mucoprotein layer termed fibrinoid which provides a continuous coating of the trophoblast in mice and was thought to mask MHC antigens (Kirby et al. 1966). Enzymatic treatment of trophoblast to remove this layer was shown to restore its immunogenicity (Currie et al. 1968), although these results have not been confirmed (Simmons et al. 1971; Jenkinson and Billington 1974; Searle et al. 1975). However, the observation that the fibrinoid layer does not occur in all species (Tai and Halasz 1967; Wynn 1969) suggests that its role in the maintenance of pregnancy may be minimal.

Later evidence indicated that a more cogent explanation for the low immunogenicity of trophoblast may be that it either lacks or has a low expression of MHC antigens at the maternal-foetal interface (Siegler and Metzgar 1970; Goodfellow et al. 1976; Faulk and Temple 1976; Faulk et al. 1977). Unfortunately, initial

investigations into this problem were hampered in a number of ways. The techniques used to look for MHC antigens were often of low sensitivity and specificity. Furthermore, instead of looking for antigens on trophoblast cells in situ in sections of whole placentas, the placental architecture was often disrupted, or else placental cells were grown in vitro and then assayed (Chatterjee-Hasrouni and Lala 1981; Wegmann 1981).

Current evidence indicates that, in the mouse, paternal H-2K but not Ia antigens are present on trophoblast cells at the maternal-foetal interface, although the density of these antigens may be low (Wegmann 1981; Chatterjee-Hasrouni and Lala 1982). Likewise in the human, HLA A, B and C but not DR antigens have been detected on some, although not all, trophoblast cells in direct contact with maternal tissues (Sunderland et al. 1981). This labelling is specific rather than via the Fc region of the antibody because labelling of mouse trophoblast is also observed with $F(ab')_2$ fragments (Raghupathy et al. 1981). The finding of paternal MHC antigens on trophoblast directly accessible to the maternal circulation is perhaps not surprising because they have been found on both choriocarcinomas (Shaw et al. 1979) and hydatiform moles (considered to be a precursor of choriocarcinoma) (Yamashita et al. 1979) which are also able to evade the maternal immune response.

What is the significance of these findings? Firstly, the presence of paternal MHC antigens on trophoblast renders these cells liable to immunological rejection by the mother. Secondly, if maternal MHC antigens are also present on trophoblast, according to Zinkernagel and Doherty (1979), any infected cells displaying viral antigen as well as (in the mouse) maternal H-2K or H-2D antigens should be destroyed by specific maternal anti-viral cytotoxic T-cells. Obviously, the first proposal does not occur and there is no evidence for the second. Thirdly, both Wegmann (1981) and Chatterjee-Hasrouni and Lala (1982) considered that the lack of Ia antigens may weaken or

eliminate the immune response against trophoblast but this concept is not compatible with other evidence. For example, Klein (1978) noted that point mutations in only the K or D regions of the mouse H-2 complex were sufficient to induce allograft rejection. In fact, these mutations were actually defined by skin graft rejection responses. Furthermore, it is unlikely that the lack of Ia antigens on the trophoblast would preclude the induction of an effective maternal immune response because the stimulation of a functional transplantation immunity against foetal antigens prior to conception does not affect the course of subsequent pregnancies (Medawar and Sparrow 1956).

In summary, if paternal MHC antigens are displayed on the surface of trophoblast cells and are accessible to the maternal immune response, mechanisms must exist which prevent the expression of transplantation immunity against the foeto-placental unit. As will be discussed in the next section, some of the mechanisms proposed to govern this suppression or blocking of maternal immune reactivity in the placenta could also have a profound effect on the expression of anti-viral immunity.

1.2.3 Does maternal immuno-suppression explain the non-rejection of the allogeneic foetus?

1.2.3.1 The evidence for a pregnancy-associated systemic immuno-suppression.

A wide range of both specific and non-specific immuno-suppressive mechanisms have been observed in pregnant subjects. Cells able to suppress various immune responses have been detected in pregnancy by a number of authors (Hamilton and Hellstrom 1977; Smith and Powell 1977; Clark and McDermott 1978; 1981; Chaouat and Voisin 1979; Suzuki and Tomasi 1979), and appear to be of at least 3 types:

a) Thymus-derived lymphocytes which inhibit maternal reactivity against cells bearing paternal antigens (Smith and Powell 1977; Chaouat and Voisin 1979). Suppressor T-cells have also been shown to non-specifically decrease antibody responses during pregnancy (Suzuki and Tomasi 1979).

b) A nylon-adherent, non-T-cell present in a B-cell enriched, macrophage-depleted fraction which also non-specifically decreases antibody synthesis (Suzuki and Tomasi 1979).

c) A non-T-cell which non-specifically suppresses the generation of effector cells of transplantation immunity and develops peak activity at the time of implantation (the authors consider this to be the time of "grafting") (Clark and McDermott 1978; 1981; Clark et al. 1980). These cells tend to be located in the lymph nodes which drain the uterus and act selectively against the generation of cytotoxic T-cells (Clark et al. 1980). They are thought to be hormonally triggered and are found in both syngeneic and allogeneic pregnancies, as well as mice pseudopregnant to sterile males (Clark and McDermott 1981).

There is a considerable amount of evidence that lymphocyte responses may also be depressed by factors present in the serum of pregnant subjects. In both animals and humans maternal serum has blocked the Mixed Lymphocyte Reaction (MLR) (Kasakura 1971; Curzen et al. 1972; Jenkins and Hancock 1972; Leikin 1972; Revillard et al. 1972; Robert et al. 1973; Harrison 1976; Herva and Jouppila 1977; Pavia and Stites 1979), decreased lymphocyte responses to mitogens (Walker et al. 1972: St. Hill et al. 1973; Hsu 1974; Yu et al. 1975), inhibited lymphokine production by maternal leukocytes in response to paternal antigens (Youtananukorn and

Matangkasombut 1973; Rocklin et al. 1976), abrogated the inhibitory effects of maternal lymphocytes on embryonic cell colony formation (Hellstrom et al. 1969), and exhibited a strong anti-inflammatory effect (Stimson et al. 1977). These inhibitory factors may include hormones (Hagen and Froland 1972), antibodies (Pence et al. 1975; Taylor and Hancock 1975; Rocklin et al. 1976; Stimson et al. 1979); circulating immune complexes (Masson et al. 1977) and pregnancy zone protein (Birkeland et al. 1979).

Although there is no doubt that the suppression of various immune responses by pregnancy serum or suppressor cells can be detected by in vitro tests, it is not clear whether these phenomena play a similar immuno-suppressive role in vivo. This is especially the case with the mechanisms which would be expected to display a generalised systemic immuno-suppression in the mother (e.g., hormones - Hagen and Froland (1972); pregnancy zone protein - Birkeland et al. (1979); suppressor cells - Clark and McDermott (1981)). Although Mims (1976a) considered that the increase in the level of corticosteroid hormone production during pregnancy may decrease the pregnant subject's ability to control infections, this is not supported by other workers who suggest that pregnancy is associated with a relatively normal immune reactivity against tumours, experimental grafts and infections (Faulk and McIntyre 1981, Siiteri and Stites 1982). Those examples where pregnancy has been associated with an increased susceptibility or severity of disease (Knox 1950; Weinstein et al. 1951; Dalldorf and Gifford 1954: Siegel and Greenberg 1955; Campbell 1960; Farber and Glasgow 1968; Rao 1972; VanZon and Eling 1980; Khuroo et al. 1981), may be explained by the added influence of nutrition, environment, genetic make-up, and physical stresses on the pregnant subject (Banatvala 1977).

Even those mechanisms which are considered to only inhibit maternal responses against paternal antigens

displayed on the trophoblast (e.g., "blocking" antibodies - Rocklin et al. 1976; Stimson et al. 1979) have to account for 2 characteristics of pregnant hosts noted by Beer and Billingham (1979): firstly, the pregnant host can act against paternal skin grafts or cells without prejudicing foetal survival; secondly, the foetus is refractory to adoptive immunisation of the host by a specifically immunised syngeneic donor.

In summary, although numerous cell types and serum factors from pregnant subjects are able to suppress immune responses in vitro, observations of maternal immuno-competence during pregnancy suggest that such suppressors are not functional in vivo. It is tempting to speculate that many of these mechanisms, rather than being crucially involved in causing the maternal hyporeactivity against the foetus, occur only as a consequence of the pregnant state. This is especially the case with phenomena such as the suppressor T-cells described by Chaouat and Voisin (1979) which cannot be detected in primiparous animals and are only observed after several pregnancies.

1.2.3.2 The evidence for immuno-suppression in the micro-environment of the placenta

Siiteri and Stites (1982) considered that the local release of an immuno-suppressive factor in the placenta with non-specific effects on maternal cellular immunity could provide an explanation for the lack of maternal reactivity against foetal trophoblast. One group of candidates for such factors would be the hormones produced by the foetus or placenta. In humans these can be classified into three groups (Biggers 1980):

a) hormones produced by the placenta independently of the embryo and foetus (human chorionic gonadotropin, human chorionic somatomammotropin and progesterone);

b) hormones produced solely by the foetus (corticosteroids); and

c) hormones that are only produced by the joint action of the foetus and placenta (oestrogens).

Siiteri and Stites (1982) suggested that progesterone in particular may be important in the evasion of the maternal immune response by the foetus because it is immuno-suppressive at the high concentrations found in the placenta (2000 to 5000 ng/g) but does not influence lymphocyte function at the lower serum concentrations (100 to 150 ng/ml). The demonstrable immuno-suppressive effects of progesterone were considered to include:

a) anti-inflammatory and graft sparing effects. Implants containing progesterone prolonged allogeneic or xenogeneic skin grafts, although these effects were localised around the implant (Siiteri et al. 1977);

b) inhibition of human and murine lymphocyte activation and the generation of killer T-lymphocytes;

c) inhibition of certain macrophage functions and activity;

d) prevention of immune cell ingress into the uterus.

Siiteri and Stites (1982) noted that, although some of these properties are shared with other sex steroids and glucocorticoids, it is the selective high concentration of progesterone in the placenta which affords immuno-suppression. If high concentrations of hormones produced by the foeto-placental unit do exert a local suppressive effect on the maternal immune response against foetal antigens, maternal anti-viral immunity

may also be compromised in the placenta. This could result in the persistence of virus in the placenta and may even facilitate foetal infection. This may explain the observations of Fox (1977) who noted that the placenta is a preferential site for virus localisation in many diseases.

1.2.4 Phagocyte function during pregnancy

Cells of the macrophage-monocyte lineage are important for the clearance of virus during infections (McFarland 1974; Doherty and Bennink 1981). During pregnancy, although the numbers of circulating monocytes and neutrophils are increased (Plum et al. 1978), there is a decrease in the migratory, chemotactic, phagocytic and antigen handling capacity of these cells (Bjorksten et al. 1978; Nicklin and Billington 1979; Senelar and Bureau 1979; Bjorksten 1980). These impaired functions may result in not only impaired inflammatory responses but also increased susceptibility to infection (Bjorksten 1980).

1.2.5 Summation of Chapter 1.2

Investigations of the reasons why the allogeneic foetus is not rejected by its mother are complicated because it is difficult to perform experimental manipulations of the pregnancy in vivo. Nevertheless, a large amount of in vitro experimentation has provided numerous possible explanations for the paradox of pregnancy, although it is difficult to assess whether these phenomena are functional in vivo or whether they have no relevance to foetal survival but occur as a consequence of the pregnant state. In a teleological sense, any explanation which implicates mechanisms which would prejudice the ability of the mother to combat infection or neoplastic growth is extremely poor and, as Siiteri and Stites (1982) noted, does not fit in with the observations of maternal immuno-competence during pregnancy. Thus, it seems unlikely that the

mechanisms concerned with the maintenance of pregnancy will be shown to influence maternal immunity to infection, except possibly in the micro-environment of the placenta.

CHAPTER 1.3

REVIEW: THE PASSIVE TRANSFER OF IMMUNITY FROM A MOTHER TO HER PROGENY

The passive acquisition of maternal antibodies is essential for providing a neonate with protection against infection until its own active immune systems are developed. Depending on the species, this antibody transfer may be either pre-natal, post-natal or both. In ungulates, for example, almost all antibody is obtained post-natally, after ingestion of colostrum. On the other hand, in primates, passively acquired antibody is mainly transferred pre-natally, across the chorioallantoic placenta. In mice, both pre-natal transfer across the yolk sac and post-natal transfer occur (Miller 1966), although the colostral and milk antibody may provide a more effective protection (Bruce-Chwatt and Gibson 1956; Brambell 1966; Iida et al. 1973; Nejamkis et al. 1975; Breniere and Viens 1980). Porter (1976) suggested that this variation between species may be due, at least in part, to their different types of placentation. Antibody may not transfer to the foetus in pigs and ruminants because of their epitheliochorial placentation where several epithelial layers are interposed between the maternal and foetal circulations. In the haemochorial form of placentation observed in humans and mice, there are fewer layers between the circulations and this possibly facilitates pre-natal antibody transfer.

1.3.1 Pre-natal antibody transfer to the foetus

The maternal antibody class usually transferred pre-natally is IgG. The mechanisms involved in its transport to the foetus have been studied in a number of species. Brambell (1966) initially proposed that the transfer of antibody across the rabbit yolk sac to the foetus occurred in a number of steps. The yolk sac endodermal cells were thought to take up extracellular proteins into pinocytic vesicles formed by invaginations of the cell membrane. IgG was thought to be bound to a specific receptor on the cell wall which protected it against enzymatic degradation. The vesicles then transported the IgG to the foetal side of the cell and released it into the foetal circulation. This was

modified by Wild (1975) who suggested that the IgG is attached to receptors on the outside of the yolk sac endoderm or, in humans, the syncytiotrophoblast, and taken up by micropinocytic "coated" vesicles, so called because of characteristic electron dense ridges observed on their cytoplasmic surface that involve the protein clathrin (Ockleford and Whyte 1977; Huesner 1980). These "coated" vesicles have not been observed to fuse with lysosomes and therefore provide protection for the IgG as it moves across to the foetal side of the cell. Although it is generally accepted that receptors for the Fc end of IgG do, in fact, occur on human syncytiotrophoblast, as yet there is no evidence that the coated vesicles reach the abluminal basal plasmalemma and discharge their contents (Johnson and Brown 1981). It is of interest that IgG molecules from other species with significant pre-natal antibody transfer will bind to human syncytiotrophoblast receptors, whereas IgG obtained from cattle and sheep, with no pre-natal transfer, will not (Matre and Haugen 1978; van der Meulen et al. 1980).

1.3.2 Post-natal antibody transfer to the neonate

A description of the mechanisms involved in the post-natal transfer of maternal antibodies to the neonate is more complicated because mammalian milks differ significantly from each other and appear to be tailored for the needs of the individual species. For example, in humans, all systemic IgG is gained by the foetus before birth, and the role of colostral and milk antibodies is mainly to suppress the growth of potential pathogens in the neonatal gut, and therefore promote a balanced and desirable flora (Reiter 1981). IgA predominates in human colostrum, with 5000 mg/day being produced in the first week post-partum. The concentrations of IgG (50 mg/day) and IgM (70 mg/day) are much lower (Ogra et al. 1980). The main function of the IgA is probably to neutralise viruses, bind to antigens and toxins, and agglutinate bacteria in the

intestinal lumen (Carlsson et al. 1980). For this reason, the bulk of the IgA is not absorbed and is eventually excreted in the faeces (Ogra et al. 1977). The IgA present in human colostrum is in the secretory 11S form (Tomasi et al. 1965), which is especially resistant to variations in pH or degradation by enzymes (Lindh 1975), and is therefore more suited to the gut environment than other antibody classes. It is of interest that the antibody activity in human colostrum and milk is mainly directed against the commoner alimentary pathogens, notably Eschericia coli and the enteroviruses (Jelliffe 1976). This may be because the specific IgA-producing plasma cells in the breast tissue originate from the Peyers patches. Following exposure to antigens from the gut, the bulk of these cells move into the systemic circulation and selectively home to the mammary tissues and other mucosal surfaces (Roux et al. 1977; Mellander et al. 1981). A similar mechanism involving the migration of plasma cells from the broncho-pulmonary lymphoid tissue has also been invoked to explain the presence of antibodies to respiratory syncytial virus in human milk (Fishaut et al. 1981).

A contrast to the situation in humans is seen in pigs, cattle and horses, where no significant pre-natal antibody transfer occurs and the major role of colostrum is therefore to provide the neonate with high serum levels of IgG to enhance its systemic immunity (Bullen 1981). Antibody, along with other macromolecules, is non-specifically absorbed from the colostrum in the intestine but this only occurs in the first 20-24 hours after birth (Jeffcott 1974). High concentrations of antibody, especially IgG, are present in colostrum during this period. For example, in pig colostrum, 63.6% of whey protein is immunoglobulin, of which 79.7% is IgG, 14.1% is IgA and 6.3% is IgM. All 3 classes are absorbed by the piglet so that the post-colostral serum concentrations of IgG and IgM are similar to the adult levels, and the concentration of

IgA generally exceeds the adult (Porter 1969). A feature of lactation in the pig, however, is the rapidly changing antibody profile in colostrum which occurs 24 hours after birth. The relative antibody concentrations then resemble those in human colostrum, with IgA replacing IgG as the predominant class. These changes are presumably to enhance the intestinal defenses against infection (Porter 1976).

The post-natal transfer of maternal antibody to the neonatal mouse and rat is dependent upon different mechanisms to those in the pig. In mouse colostrum, IgA is present at a concentration of 0.26 mg/ml, IgM at 0.07 mg/ml, IgG1 at 0.24 mg/ml, IgG2a at 0.19 mg/ml and IgG2b at 0.03 mg/ml. IgG3 is not detectable (Guyer et al. 1976). Neither IgA nor IgM are absorbed by the neonate (Fahey and Barth 1965; Guyer et al. 1976), suggesting that their primary protective role is in the intestinal lumen. On the other hand, between 30% and 50% of colostral IgG is selectively absorbed (Guyer et al. 1976). The mechanism involved in this selective absorption appears to be similar to that responsible for the pre-natal transfer of IgG to the foetus described earlier in this Chapter. Receptors for the Fc end of IgG are present in the mouse intestine. The affinities of the different IgG sub-classes for the receptor are

$$IgG2a = IgG1 > IgG2b \simeq IgG3$$

(Guyer et al. 1976). In the young rat, these receptors are only located in the proximal region (duodenum and jejunum) of the small intestine. Furthermore, they are pH-dependent and will only bind IgG at the acid pH found in the gut (Wild 1981). Rodewald (1980) proposed that after binding to the receptors, the IgG is transported from the luminal surface to the abluminal surface by "coated" vesicles and is therefore protected against intracellular degradation. Exposure of the receptors to the serosal plasma which is at a neutral pH would probably effect the release of the IgG.
It is of interest that these receptors cannot be detected

in the rat intestine after 20 days of life (Wild and Richardson 1979), which coincides with the time that the neonate loses its ability to absorb IgG from the gut (Halliday 1956).

1.3.3 Other mechanisms involved in the passive protection of the neonate by colostrum

Passive protection of the neonate by the mother is not only mediated by antibody, although this is extremely important. In humans, a multifactorial defense system against infection is present in colostrum and milk and includes lactoferrin, transferrin, lactoperoxidase, lysozyme, "bifidus" factor, lipid-associated staphylococcal resistance factors, monoglycerides with anti-viral activity, components of the complement system, interferon, lymphokines, chemotactic factors and IgA-stimulating factors (Ogra et al. 1980; Bullen 1981). There may also be a significant immune cell presence. At one day post-partum, human colostrum has been shown to contain 2.1×10^6 macrophages/ml, 5.6×10^5 neutrophils/ml and 2.4×10^5 lymphocytes/ml, although these concentrations decrease with time (Ogra et al. 1980). The macrophages may be important in the defense of both the breast tissue and the neonatal gut (Pittard et al. 1977; Pitt 1979; Mellander et al. 1981). The lymphocytes, of which 50% are T-lymphocytes (Diaz-Jouanen and Williams 1974), are responsive to a variety of mitogens and microbial antigens in vitro (Ogra and Ogra 1978). Although evidence exists that certain antigen-specific lymphocyte reactivities may be transferred from the mother to her neonate by suckling (Mohr 1973; Ogra et al. 1977; Schlesinger and Covelli 1977), it is not clear whether this represents the uptake of these sensitised cells, or the absorbtion of molecular mediators of immunity such as transfer factor (Diaz-Jouanen and Williams 1974).

CHAPTER 1.4

INTRODUCTION TO THE THESIS

The discovery that viral pathogens can cause damage to the foetus *in utero* was made just over 40 years ago by Gregg in 1941. Since then, efforts have been made to elucidate the various mechanisms utilised by viruses in the pathogenesis of foetal infections. However, unlike most other host-parasite interactions, the pathogenesis of infections during pregnancy is complicated because:

a) two hosts, the mother and the foetus, are present, and each has a different genetic make-up and different level of immune maturation and responsiveness;

b) the placenta, an organ unique to pregnancy, is present and may provide another target for the pathogen;

c) the maternal immune response to infection may be compromised by the co-existence of mechanisms which serve to prevent rejection of the allogeneic foetus and its placenta.

The aim of this thesis was therefore to contribute to the understanding of the immunology and pathogenesis of *in utero* virus infections using the laboratory mouse as an experimental host. Originally, it was intended to use Akabane virus in this study because of its importance in causing foetal teratogenesis and wastage in farm animals. However, attempts to establish foetal infection in the pregnant mouse failed, probably because the virus did not produce a viraemia in the mother (unpublished results). It was therefore decided to use 2 serologically related alphaviruses, Semliki Forest virus (SFV) and Ross River virus (RRV) which, in preliminary studies, produced highly reproducible but different syndromes of foetal infection. There have been no reports of the effects of either experimental or natural infections with SFV during pregnancy.

On the other hand, RRV has been shown to establish in the foetus and placenta following the experimental infection of pregnant mice (Aaskov et al. 1981a), and may occasionally cross the placenta during natural disease in humans (Aaskov et al. 1981b). The value of using laboratory models to study the pathogenesis of in utero infection was emphasized by Fuccillo and Sever (1973) who noted,"Further development of animal models of congenital viral infections will help contribute to our knowledge of the number of variables which influence the frequency of congenital infections and their pathogenic processes", and Mims (1976b) who, in discussing the problems of understanding the pathogenesis of viral infections of the foetus, noted that "As in many aspects of medicine, progress is more rapid when a laboratory animal system is available. Naive extrapolations to the human situation are rarely justified but the animal model at least gives vital information about the sort of thing that can happen and, compared with clinical research progress can be rapid". Thus, although the use of a laboratory model and artificial infection regimens constitutes a rather contrived situation, it is hoped that the results obtained from such a controlled and reproducible methodology may be of value in leading to a greater understanding and possibly improved control of natural in utero viral infections in both human and veterinary medicine.

CHAPTER 2

MATERIALS AND METHODS

2.1 Viruses

The reference strains of alphaviruses used in the present study are listed in Table 2.1. Both RRV and SFV strain A7 are uniformly lethal for neonatal mice but do not cause clinical signs in adult or pregnant mice. SFV strain 25639 is lethal for both neonatal and adult mice. All stock viruses were prepared as 10% ($^{w}/v$) suckling mouse brain suspensions in normal saline and were stored at -65^{o}C.

2.2 Mice

All mice were bred by the Animal Breeding Establishment in the John Curtin School of Medical Research. Outbred mice used for immune ascites fluid preparation were multi-coloured Walter and Eliza Hall Institute strain mice. The CBA/H inbred strain was used for all experiments except where specified in Tables 4.1 and 5.6. Adults were always between 7 and 10 weeks of age. The onset of pregnancy (gestation day 0) was taken as the day of observation of a vaginal plug following mating. All non-pregnant mice used for comparisons with pregnant mice were age-matched CBA/H females.

Mouse inoculations were performed intraperitoneally (i.p.), intravenously (i.v.) and subcutaneously (s.c.).

2.3 Collection and preparation of tissues for virus assay

At harvest, mice were anaesthetised with chloroform, then exsanguinated. Tissues were aseptically removed, weighed and labelled, and stored at -65^{o}C. Subsequently, they were ground in a measured volume of gelatine saline using a mortar and pestle with sterile sand, spun at 850g for 5 min., and the supernatant was removed for virus assay.

Table 2.1 Viruses

Virus	Original strain designation	Source	Passage History
Ross River	T48	Dr. R.L. Doherty[1]	13 smb[2]
Semliki Forest	A7	Prof. H. Smith[3]	9 smb, 2 cec[4]
Semliki Forest	25639[5]	RFVL[6]	15 amb,[7] 5 smb

1. Queensland Institute of Medical Research, Brisbane, Australia.
2. smb = suckling mouse brain.
3. University of Birmingham, England.
4. cec = chicken embryo cells.
5. This strain of SFV was only used in Chapter 3, Table 3.5, and is termed "virulent" SFV.
6. RFVL = Rockefeller Foundation Virus Laboratories, New York, U.S.A.
7. amb = adult mouse brain.

Serum, collected after centrifugation of the clotted blood, was stored at -65°C until use.

2.4 Cell culture

Cell line

Vero cells, originally derived from the kidney epithelium of the African Green Monkey (Cercopithecus aethiops) were supplied by the tissue culture laboratory, Microbiology department, John Curtin School of Medical Research.

Virus titrations

Plaque assays were performed on confluent 24 hour Vero cell monolayers, grown in 60 mm petri dishes in Medium 199 (Gibco) with 5% bovine serum, 100 ug/ml penicillin, 100 ug/ml streptomycin and 100 ug/ml neomycin, and incubated in a humidified atmosphere with 5% CO_2 at 37°C. After draining, replicates of 2 or 3 monolayers were inoculated with 0.1 ml of the appropriate serial virus dilution. Virus was allowed to adsorb to the monolayer for 1 hour at 37°C, after which 5 ml of an agar overlay was added to each plate.

The overlay consisted of Earles Salts Solution (Gibco) with 0.5% lactalbumin hydrolysate, 0.1% yeast extract, 0.1% bovine plasma albumin (fraction V) and 0.75% Difco Bacto agar. In addition, all overlays contained 100 ug DEAE dextran/ml, 0.05% sodium bicarbonate, 2% bovine serum, 0.02M HEPES buffer and antibiotics.

Plates were incubated at 35°C in a non-gassed, non-humidified incubator for 2 days with SFV, or 3 days with RRV, when 3 ml of a 0.01% neutral red in saline solution was added. After re-incubation at 35°C overnight, plaques were counted.

Virus titres are expressed as the number of plaque forming units per ml (pfu/ml) or per gram (pfu/gm) as appropriate.

2.5 Fractionation of antibodies from serum

IgG subclasses were fractionated from serum by affinity chromatography using a column made from staphylococcal protein A covalently linked to sepharose CL-4B (Pharmacia), according to a modification of the methods described by Ey et al. (1978) and Seppala et al. (1981). The IgG binds to the protein A by its Fc end whereas all other antibody classes are washed through the column. The IgG can then be eluted by lowering the pH (Forsgren and Sjoquist 1966; Hjelm et al. 1972).

Approximately 2 ml of serum was diluted with 4 ml of 0.14M sodium phosphate buffer pH 8.0, then applied to a 5 ml protein A-sepharose column equilibrated in the same buffer at 4°C. A linear pH gradient made from 150 ml of 0.14M sodium phosphate buffer pH 8.0 and 150 ml of 0.1M citric acid buffer pH 2.7 was then applied. The flow rate was 30-35 ml per hour. Fraction size was approximately 2.9 ml. All fractions with a pH of less than 5.0 were taken into 0.8 ml of 1M Tris-HCl pH 9.0 to neutralise the acidity. After fractionation, the column was re-equilibrated to pH 8.0 and used again.

The protein concentration in each fraction was assayed using the micro-method of Rylatt and Parish (1982). 15 ul of test sample and 85 ul of normal saline were added to 100 ul of a 0.06% solution of Coomassie Blue (Sigma) in 1.9% perchloric acid in wells of a 96-well round bottom plate (Linbro). After 2 min. the optical density of the solution was read in a Dynatech ELISA spectrophotometer, with test wavelength 630 nm and reference wavelength 405 nm. The protein concentration of the sample is proportional to the optical density and is assessed from the linear section of an optical density versus serum albumin standard curve.

The location of each protein peak eluted from the column was then found from a plot of optical density versus fraction number. The appropriate fractions were pooled, concentrated to approximately 1-2 ml by vacuum dialysis, dialysed against normal saline, and stored at - 20°C.

2.6 Gel diffusion test

The various immunoglobulins present in the protein peaks eluted from the sepharose-protein A column were identified by gel-diffusion using rabbit-anti-mouse antisera (Bionetics) specific for mouse IgA, IgM, IgG1, IgG2a, IgG2b (all generously donated by Dr. Ian McKenzie, Melbourne University), and IgG3.

Three ml. of a 1% purified agar (Oxoid) solution with 1% NaCl was layered onto a clean glass microscope slide. Approximately 10 ul of undiluted antisera or the appropriate protein peak was added to adjacent, small 2 mm holes punched into the agar layer. The slide was incubated overnight in an humidified atmosphere at room temperature to allow formation of precipitin lines which were only observed when the immunoglobulin present in the protein peak was of the correct specificity to allow complexing with the antiserum.

2.7 The haemagglutination-inhibition (HI) test

Anti-viral antibody was assayed in the HI test using the Takatsky microtechnique (Sever 1962) adapted to the general methods of Clarke and Casals (1958). SFV haemagglutinin was prepared by sucrose-acetone extraction of infected infant mouse brain. RRV haemagglutinin was prepared in the same way from eviscerated infant mouse carcasses.

Sera were extracted with acetone and ether to remove non-specific lipid inhibitors of haemagglutination. Extracted sera were adsorbed with gander erythrocytes prior to testing. The test was performed using between 4 and 8 units of haemagglutinin, and the end-point was taken as the reciprocal of the highest dilution of serum which resulted in 50% haemagglutination.

2.8 Immunofluorescence (IF)

An indirect IF method was used for the detection and location of viral antigens in frozen tissue sections of the foetus and placenta. Eight micron sections were cut in a cryostat, mounted on gelatin-treated microscope slides, dried at room temperature, fixed for 5 min. in acetone at -20°C, then stored at -20°C until staining.

Sections were thawed at room temperature, overlaid with an unlabelled mouse anti-virus serum, then incubated at 37°C for 30 min. in a humidified chamber. Sections were washed three times in phosphate-buffered saline, 3 min. per wash, then overlaid with a FITC-conjugated rabbit anti-mouse IgG (Miles-Yeda). After incubation for a further 30 min. at 37°C in a humidified chamber, the sections were washed 3 times in phosphate-buffered saline, air dried and mounted in 90% glycerol in borate-buffered saline pH 9.0. They were examined using a Zeiss fluorescent microscope.

2.9 Preparation of immune ascites fluid

Ascites fluid was prepared by the method of Tikasingh et al. (1966), using sarcoma 180/TG cells. Mice were given 0.2 ml i.p. of a 1:1 mixture of undilute virus stock: Freunds complete adjuvant each week for 5 weeks. Three days before the last injection 0.1 ml of a 10% sarcoma cell suspension was given i.p. Ascites fluid was collected between 10 and 30 days later. Normal ascites fluid was prepared from uninfected mice.

2.10 Preparation of anti-thymocyte serum (ATS)

ATS was prepared by the method of Levey and Medawar (1966). Rabbits were given i.v. injections of 2×10^8 and 10^9 viable CBA/H mouse thymocytes 14 days apart, and were exsanguinated 9 days later. Normal rabbit serum (NRS) was used as a control for the ATS.

Both sera were stored at -20°C. ATS treatment on its own did not affect the course or outcome of a normal mouse pregnancy.

2.11 The lymphocyte stimulation test (LST)

Antigens and mitogen

Viable virus preparations were used as antigens because Griffin and Johnson (1973) showed that they induced better proliferation in the LST than inactivated virus. Antigens were obtained by infecting confluent Vero cell monolayers with RRV or SFV at a multiplicity of infection of 1:1. The infected monolayer was grown in medium Fl5 (Gibco) with 5% foetal calf serum (fcs) (Flow Laboratories) until extensive cytopathic effect was observed. The supernatant was then removed, clarified at 850g for 10 min. and stored at -20°C.

Concanavalin A (Con A), derived from the jack bean (Canavalia ensiformis), is a T-cell mitogen (Schechter 1980), and was always used as a positive control.

LST methodology

Variables such as incubation time, cell numbers and antigen dosage were initially optimised using spleen and para-aortic lymph node cells from infected, pregnant mice. The test used in the present study was as follows: Single cell suspensions of spleen or lymph node were obtained by pressing the organs through a fine stainless steel wire mesh into medium Fl5 with 5% fcs. Lymphocytes were isolated from the spleen cell preparation by centrifugation in a Ficoll-Isopaque gradient at 20°C for 20 min. at 2000g, according to the procedures of Davidson and Parish (1975). Lymph node cells were used unseparated. The cell suspensions were washed twice in Fl5 and finally resuspended to 2×10^6 cells/ml in Fl5 plus 5% fcs and 10^{-4}M of 2-mercaptoethanol (Sigma).

100 ul of the suspension was dispensed into wells of a 96-well flat-bottomed tissue culture plate (Linbro) along with 50 ul of the appropriate antigen or Con A. This corresponded to approximately 6.5×10^5 pfu of both RRV and SFV. Con A was used at a concentration of 10 ug/ml. Between 4 and 8 replicates were used for each assay. Plates were incubated at $37^{o}C$ for 72 hours in a gas mixture of 10% CO_2, 7% O_2 and 83% N_2.

The extent of lymphocyte proliferation in response to antigen or mitogen was assessed by adding 0.6 uCi of methyl-tritiated thymidine (^{3}HTdR) (Amersham) in 50 ul of Fl5 with 5% fcs to each well 6 hours before the end of incubation. At harvest the cells from each well were washed out with distilled water onto Whatman GF/A filter paper using a multiple sample harvester (Dynatech). Each sample was counted in a Packard model 3320 scintillation spectrometer. Results were expressed either as counts per minute (cpm) or as a stimulation index (SI) which was defined as follows:

$$SI = \frac{\text{cpm with stimulant}}{\text{cpm without stimulant}}$$

Stimulation indices greater than 2.0 were considered to indicate a specific response against antigen (Muscoplat et al. 1974; Buergelt et al. 1977).

2.12 Measurement of T-cell mediated cytotoxicity

Target cells for the assay of anti-viral cytotoxic lymphocytes were 120-hour thioglycollate-induced CBA/H peritoneal macrophages (Mullbacher 1981). Approximately 5×10^6 macrophages were labelled with 400 uCi of ^{51}Cr (sodium chromate, 200 mCi/mg, CEA commissariat A L'energie Atomique) for 1 hour at $37^{o}C$ in a volume of 0.5 ml. They were simultaneously infected with 10 to 100 pfu/cell of the appropriate virus. After incubation, the cells were washed 3 times to remove excess label, and 2×10^4 cells were added in 100 ul of Fl5 with 5% fcs and 10^{-4}M 2-mercaptoethanol

to wells of a 96-well round-bottom tissue culture plate (Linbro).

Single cell suspensions of spleen or lymph node effector cells were added in 100 ul amounts to the infected target macrophages so that effector:target ratios of 30:1, 10:1, 3.3:1 and 1.1:1 were achieved. All ratios were performed in triplicate. To enable a calculation of the specific anti-viral lysis to be performed, effectors were also assayed for their cytocidal activity on target cells that were labelled but not infected. Controls were infected and uninfected targets with medium only, i.e., no effector cells (medium release), and infected and uninfected target cells with 1% Triton-X100 (maximal release). After incubation for 6 hours at $37^{o}C$ in an atmosphere of 10% CO_2, 7% 0_2 and 83% N_2, 100 ul of supernatant from each well was assayed for the release of ^{51}Cr from the target cells in a Beckman gamma 9000 spectrometer.

The % lysis for a particular assay was calculated by the formula:

$$\% \text{ lysis} = \frac{\text{test sample release - medium release}}{\text{maximal release - medium release}} \times 100$$

Medium release was usually between 10 and 25%. Standard errors of the mean of each triplicate were always less than 5%.

The % specific lysis, attributable to cytotoxicity against viral antigens, was calculated by the formula:

$$\% \text{ specific lysis} = \begin{array}{l}\% \text{ lysis on infected targets -} \\ \% \text{ lysis on uninfected targets.}\end{array}$$

2.13 Depletion of T-cells by anti-Thy 1.2 plus complement treatment

T-cells were depleted from a lymphoid cell

population to assess their role in the anti-viral responses detected in the LST and cytotoxicity assay. 10^8 spleen or lymph node cells were incubated with 100 ul of anti-Thy 1.2 serum (OLAC Ltd) for 1 hour at $4^{\circ}C$. The cells were washed twice and resuspended in 1 ml of a 1:2 dilution of guinea-pig serum as a source of complement for 30 min. at $37^{\circ}C$. The cells were washed twice before assay in the LST and cytotoxicity test.

CHAPTER 3

THE EFFECT OF PREGNANCY ON THE STIMULATION AND EXPRESSION OF ALPHAVIRUS IMMUNITY IN THE MOUSE

INTRODUCTION

Doherty and Bennink (1981) summarised the major events involved in the pathogenesis of, and recovery from, an acute viral infection. These are reproduced in Table 3.1. Whether all these events occur in infected pregnant animals is, however, unclear because some mechanisms proposed to explain the non-rejection of the allogeneic foetus may also compromise the mother's anti-viral response (see Chapter 1.2), both in allogeneic and syngeneic pregnancies. The experiments described in this Chapter were designed to investigate this problem. Firstly, the effect of pregnancy on the level of *in vivo* stimulation of T-cell mediated immune responses was evaluated in mice following RRV or SFV infection using 2 *in vitro* tests. The first of these, the proliferation assay or Lymphocyte Stimulation Test (LST), measures the ability of sensitised lymphocytes to recognize and undergo clonal expansion in response to antigen *in vitro*, and is considered to be analogous to the events that occur in the T-cell populated areas of lymphoid tissues during the primary immune response *in vivo* (Mackaness 1978). In the mouse, the proliferation response involves 3 cell populations, of which 2 are T-cells and 1 is an antigen-presenting cell (APC). One T-cell is antigen-specific and is stimulated to proliferate by a H-2I region compatible APC. The other T-cell is not sensitised but is recruited into the proliferation (Tse *et al*. 1980). Although this non-specific component of the response means that measuring the level of ^{3}HTdR incorporation may not be as accurate a method of assessing the degree of cellular sensitisation to antigen as, for example, a determination of the actual number of antigen-reactive cells (Sohnle and Collins-Lech 1981), the LST has been widely used for the detection of immune responses and proved easy to perform and quantitate in the present study.

The other test used in the present study

Table 3.1 The major events involved in the pathogenesis of an acute viral infection[a]

Day after Infection	Major event
1 - 2	Localisation of virus in tissues.
2 - 4	Processing by (or infection of) stimulator cells in lymphoid tissue is followed by recruitment of precursor T-cells and clonal expansion. Similar events occur for B-cells, which differentiate into antibody-forming plasma cells.
4 - 5	Secreted antibody neutralises virus in blood and terminates viraemia.
5 - 6	Effector T-cells exit from lymphoid tissue and enter the blood, either directly (from spleen) or via the thoracic duct (from lymph nodes). These T-cells then localise in organs where virus is growing. Blood monocytes are recruited into infected tissue and differentiate to form activated macrophages. Virus is eliminated either as a result of target cells being killed directly by T-cells before assembly of infectious progeny occurs, or by activated macrophages ingesting and killing free virus. This is essentially for recovery, but may prove fatal if there is a massive synchronous destruction of functionally important cells that have not previously been physiologically compromised by infection with (for instance) a nonlytic virus.
7 on	Tissue repair and resolution of the inflammatory process.

a Reproduced from Doherty and Bennink (1981).

measures the ability of sensitised T-cells to specifically recognize and lyse infected target cells. These cytotoxic T-cells are considered to be important for recovery from viral infections (Zinkernagel and Doherty 1979; Doherty and Bennink 1981), and have been demonstrated in a number of diseases (Zinkernagel and Doherty 1979). Their function *in vivo* is considered to be the lysis of infected cells before infectious progeny virus can be produced or released (Zinkernagel and Althage 1977). For lysis to occur viral antigen must be displayed on the target cell surface and, in the mouse, the H-2K and/or D region antigens must be shared between the cytotoxic and target cells (Zinkernagel and Doherty 1979).

Experiments were then performed to ascertain whether the state of pregnancy affected the *in vivo* expression of this immunity by examining both the protective capacity of immune spleen cells from pregnant mice compared to non-pregnant mice, and the effect of pregnancy on the ability of mice to clear virus from their tissues.

RESULTS

It was considered that the best way of comparing the immune status of pregnant to non-pregnant mice following a primary infection was to use multi-point time-course assays of the different immune responses. The responses in 2 lymphoid organs were measured: the para-aortic lymph nodes (PALN) which drain the uterus (Beer and Billingham 1970), and the spleen.

The effect of pregnancy on the virus-specific lymphocyte proliferative responses.

Groups of 4 11-day pregnant mice and non-pregnant controls were harvested at different days after i.p. infection with either 2600 pfu of RRV or

7000 pfu of SFV. LST responses against specific viral antigen were assessed in the spleen and PALN of individual mice. With RRV (Figure 3.1), there was little evidence of an antigen-induced response in either the spleens or PALN of the non-pregnant mice, or in the spleens of the pregnant mice. In the pregnant PALN, a low degree of reactivity was detected with onset at day 6 post infection (p.i.). No reactivity was observed at day 10 p.i. With SFV (Figure 3.2), the responses obtained from the pregnant mice in both spleen and PALN were much higher than in the non-pregnant mice, where little or no reactivity was detected. In the pregnant spleen, reactivity to SFV antigen was first detected and peaked at day 6 p.i., but was not detected at day 10 p.i. Responses in the pregnant PALN were stronger than the spleen and were first detected at day 4 p.i., peaked at day 6 p.i., and were still detectable at day 10 p.i.

In the pregnant PALN and spleen the amount of ^{3}HTdR label incorporated in the control cultures without added SFV antigen showed a small peak at day 6 p.i. This was not observed with either organ in the non-pregnant controls.

The antigen-induced proliferation responses of lymph nodes other than the PALN from pregnant mice infected with SFV.

An experiment was performed to examine whether the proliferation response observed in the PALN following infection of pregnant mice with SFV could be detected in any other lymph nodes in these animals.

A group of 4 11-day pregnant mice were infected i.p. with 7000 pfu of SFV. At 6 days p.i., the PALN, mesenteric and axillary lymph nodes were pooled and their responses to SFV antigen were assessed in the LST (Table 3.2).

Figure 3.1 Proliferation responses to RRV antigen in the LST.

Groups of 4 11-day pregnant mice and non-pregnant controls were harvested at different times after i.p. infection with 2600 pfu of RRV. Each point represents the mean $\pm$ one standard error of the individual spleen or PALN LST responses.

□ = cpm obtained with cells cultured in medium only

● ▲ = cpm obtained with cells cultured with RRV antigen

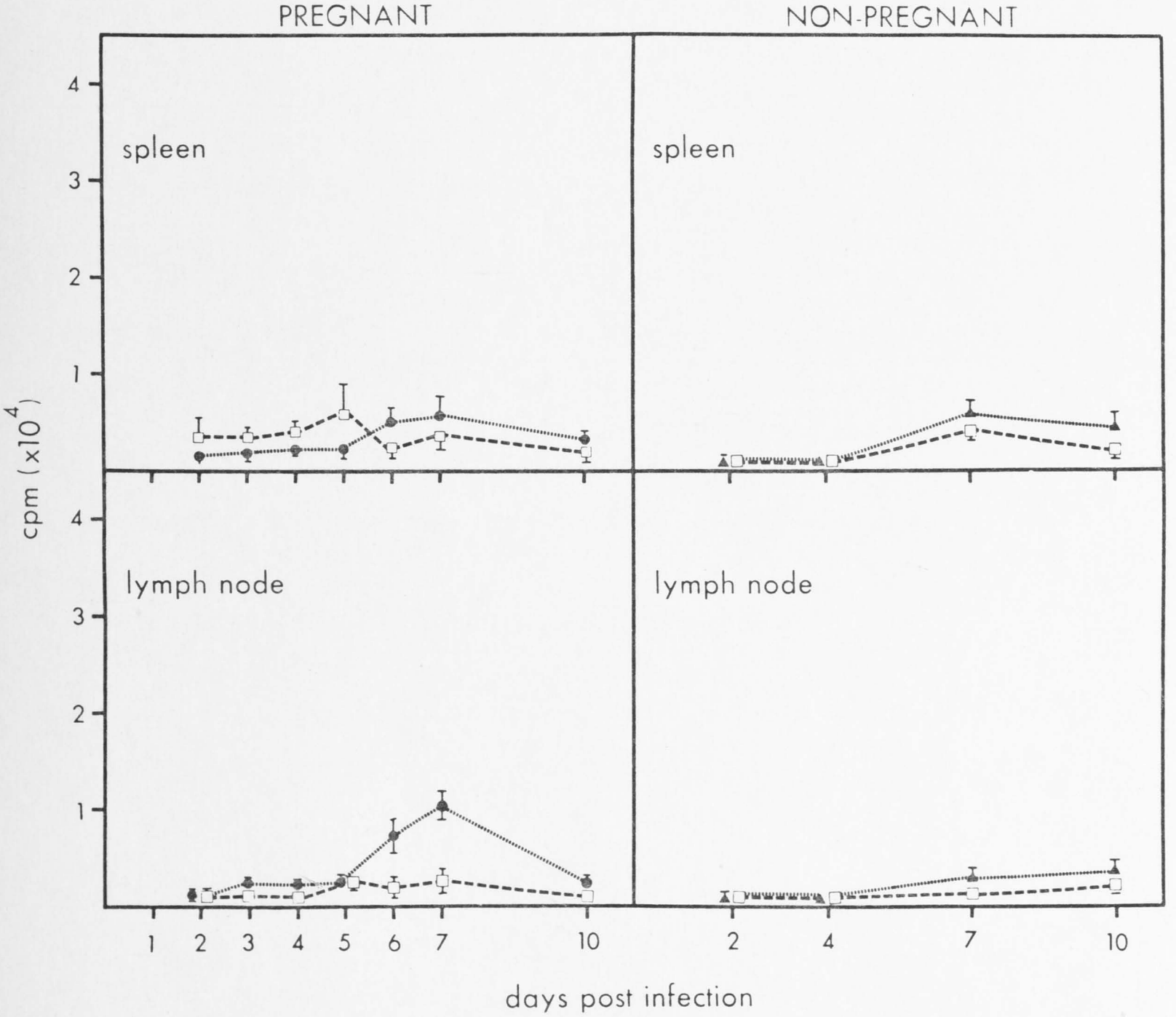

PREGNANT
NON-PREGNANT
spleen
spleen
lymph node
lymph node
cpm (x10^4)
4
3
2
1
1 2 3 4 5 6 7 10
2 4 7 10
days post infection

Figure 3.2 Proliferation responses to SFV antigen in the LST.

Groups of 4 11-day pregnant mice and non-pregnant controls were harvested at different times after i.p. infection with 7000 pfu of SFV. Each point represents the mean $\pm$ one standard error of the individual spleen or PALN LST responses.

□ = cpm obtained with cells cultured in medium only

● ▲ = cpm obtained with cells cultured with SFV antigen.

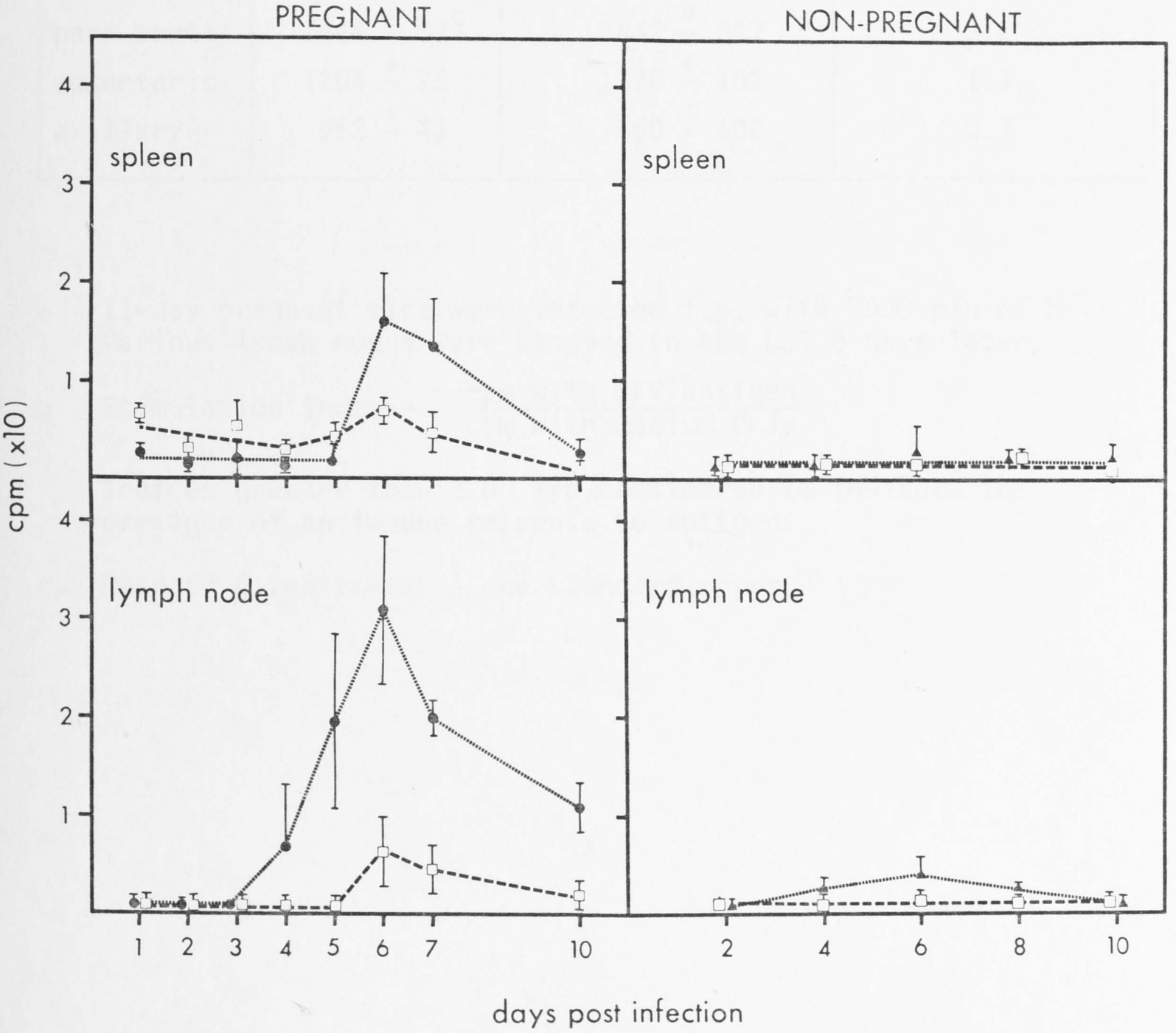

PREGNANT
NON-PREGNANT
spleen
spleen
lymph node
lymph node
cpm (x10)
4
3
2
1
4
3
2
1
1 2 3 4 5 6 7 10
2 4 6 8 10
days post infection

Table 3.2 Antigen-induced proliferation responses of various lymph nodes from pregnant animals infected with SFV[a]

Lymph node	Amount of label incorporated (cpm)		Stimulation[b] Index
	Medium only	With SFV antigen	
para-aortic	2446 ± 67[c]	7842 ± 347	3.2
mesenteric	1204 ± 73	1270 ± 102	1.1
axillary	952 ± 41	660 ± 102	0.7

a 11-day pregnant mice were infected i.p. with 7000 pfu of SFV. Various lymph nodes were assayed in the LST 6 days later.

b Stimulation Index = $\frac{\text{cpm with SFV antigen}}{\text{cpm with medium only}}$

Indices greater than 2.0 are considered to indicate the presence of an immune response to antigen.

c Mean of 6 replicates ± one standard error.

A Stimulation Index >2.0 was only observed in the PALN suggesting that these nodes were the only ones with virus-specific immune reactivity in vivo.

Identity of the PALN cells which respond to antigen in the LST.

Four 11-day pregnant mice were infected i.p. with 7000 pfu of SFV and their PALN were harvested 7 days later. The PALN cells were pooled and treated with either anti-Thy 1.2 antiserum plus complement, or complement alone. The results (Table 3.3) indicate that most of the resting activity as well as the proliferation in response to Concanavalin A and SFV antigen was abrogated by the lysis of Thy 1.2-bearing cells, suggesting that T-cells were the major cell type responding to antigenic stimulation.

The effect of pregnancy on the cytotoxic anti-viral lymphocyte response following RRV or SFV infection.

The effect of pregnancy on the time course of the primary anti-viral cytotoxic response in spleen and PALN was then assessed. Groups of 3 11-day pregnant mice and non-pregnant controls were infected i.p. with either 2600 pfu of RRV or 7000 pfu of SFV. The cytotoxic anti-viral activity in pooled spleens or PALN was assessed at various times after infection (Figure 3.3). There was little or no cytotoxic activity in either of the organs in pregnant or non-pregnant mice following infection with RRV. Following SFV infection, a strong cytotoxic response was observed in the pregnant spleen, rising at day 6 p.i., peaking at day 7 p.i. and still detectable at day 10 p.i. Unlike the results obtained with the LST, the cytotoxic activity in the pregnant PALN was weaker than that in the spleen. A low degree of anti-SFV cytotoxicity was also obtained from the non-pregnant spleen. Little or no anti-SFV activity was detected in the non-pregnant PALN.

Table 3.3 Characterisation of PALN cells[a] which respond in the LST to SFV antigen

Treatment	Amount of label incorporated (cpm)		
	Medium only	Added Concanavalin A	Added SFV antigen
Complement only	3007 ± 300[b]	15822 ± 355	8734 ± 436
Anti-Thy 1.2 serum plus complement	284 ± 59[c]	2571 ± 137[c]	1446 ± 121[c]

a PALN cells were taken from pregnant mice 7 days after i.p. infection with 7000 pfu of SFV.

b Mean ± one standard deviation from 8 replicates.

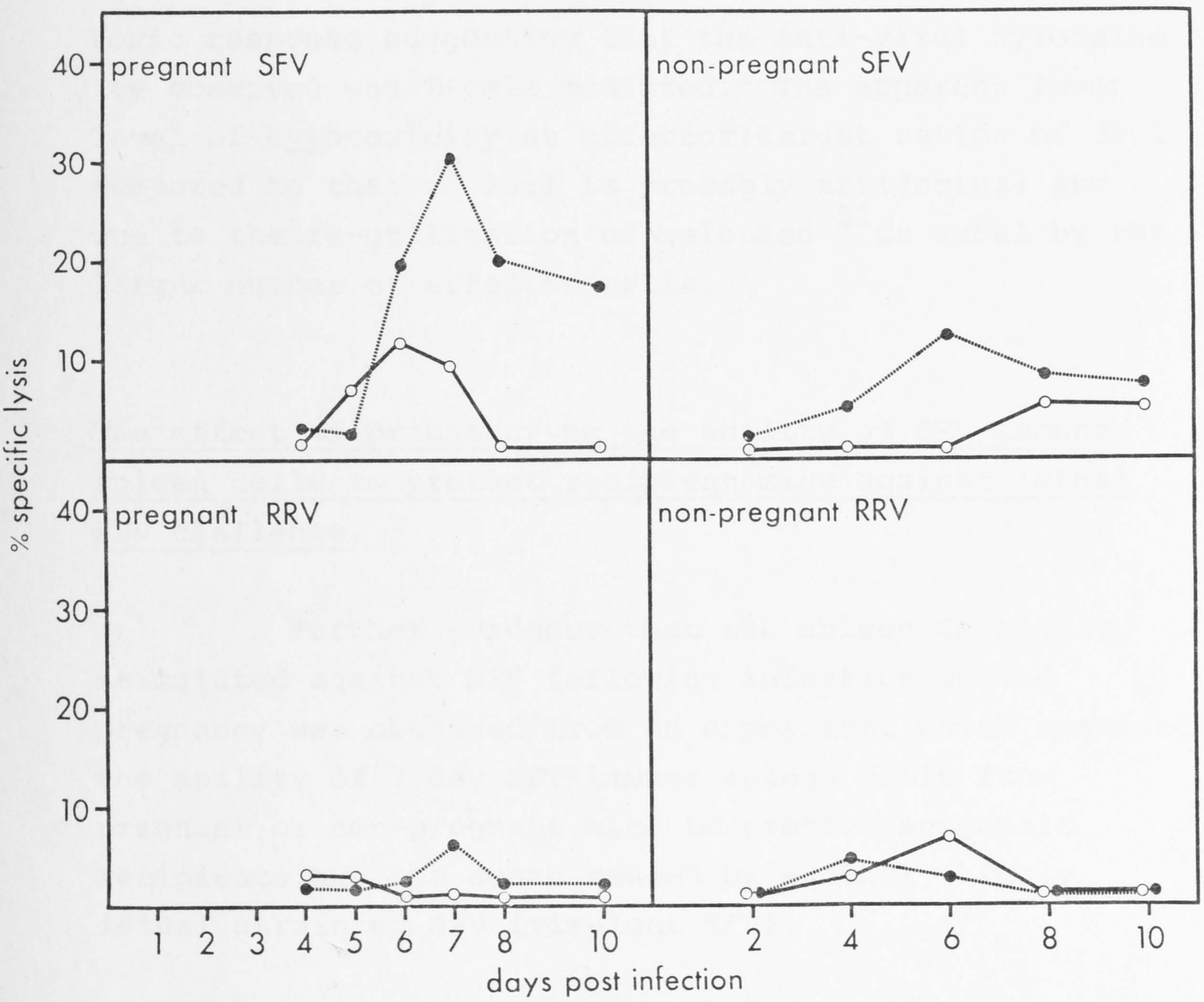
c Significantly lower than complement only value (Student's t test, $p < 0.01$).

Figure 3.3 Cytotoxic anti-viral responses in spleen and PALN of pregnant and non-pregnant mice.

Groups of 3 11-day pregnant mice and non-pregnant controls were harvested at different times after i.p. infection with either 2600 pfu of RRV or 7000 pfu of SFV. Each point represents the specific lysis obtained from pooled organs at an effector:target ratio of 30:1.

○ = specific lysis obtained with pooled PALN

● = specific lysis obtained with pooled spleens.


pregnant SFV
non-pregnant SFV
pregnant RRV
non-pregnant RRV
40
30
20
10
% specific lysis
1 2 3 4 5 6 7 8 10
2 4 6 8 10
days post infection

Identity of the spleen cells which exhibit cytotoxic anti-viral activity.

Pregnant mice were infected i.p. at gestation day 11 with 7000 pfu of SFV. At 7 days p.i., the level of cytotoxic activity was assessed in untreated, complement only treated, and anti-Thy 1.2 serum plus complement treated, pooled spleen cells (Table 3.4).

At all effector:target ratios, treatment with anti-Thy 1.2 serum plus complement abrogated the cytotoxic response suggesting that the anti-viral cytotoxicity observed was T-cell mediated. The apparent lower level of cytotoxicity at effector:target ratios of 30:1 compared to that of 10:1 is probably artifactual and due to the re-utilisation of released ^{51}Cr label by the larger number of effector cells.

The effect of pregnancy on the ability of SFV-immune spleen cells to protect recipient mice against lethal SFV challenge.

Further evidence that the spleen is highly stimulated against SFV following infection during pregnancy was obtained from an experiment which examined the ability of 7 day SFV-immune spleen cells from pregnant or non-pregnant mice to protect syngeneic recipients against death caused by another, highly lethal strain of SFV (virulent SFV).

Pregnant mice at gestation day 11 and non-pregnant controls were infected i.p. with 7000 pfu of SFV. Seven days later, the spleens in each group were pooled and different numbers of spleen cells were given i.v. to groups of 5 non-pregnant CBA/H recipients who had been infected i.p. with 1000 pfu of virulent SFV 24 hours earlier. The cytotoxic activity of each spleen cell suspension prior to administration to the recipients was, for pregnant spleen cells, 50% specific lysis, and

Table 3.4 Characterisation of the spleen cells[a] which display virus-specific cytotoxic activity following SFV infection

Treatment	% specific lysis			
	30:1[b]	10:1	3.3:1	1.1:1
Untreated	16.0	30.8	26.0	15.0
Complement only	26.3	37.9	26.6	20.4
Anti-Thy 1.2 serum plus complement	3.2	-1.4	-1.5	-0.4

a Spleens were pooled from 2 pregnant mice 7 days after i.p. infection with 7000 pfu of SFV.

b Effector:target ratio.

for non-pregnant spleen cells, 15% specific lysis, both values being obtained at effector:target ratios of 20:1. The proportion of recipients protected against death with each cell dose is presented in Table 3.5. On a cell-to-cell basis, the immune spleen cells from pregnant mice afforded much more protection to the recipients than those from the non-pregnant mice.

The effect of pregnancy on the ability of a mouse to recover from infection with RRV or SFV.

Results presented so far in this Chapter suggest that following infection with SFV, but not RRV, T-cell anti-viral reactivity is markedly enhanced during pregnancy. An experiment was therefore performed to examine whether this enhanced immunity was actually expressed *in vivo* in the pregnant mouse. This was accomplished by assessing the relative abilities of pregnant and non-pregnant mice to clear virus from their tissues.

Groups of 3 11-day pregnant mice and non-pregnant controls were infected i.p. with either 2600 pfu of RRV or 7000 pfu of SFV. Brain, muscle, spleen, liver and serum from each group were pooled at various times after infection. All tissues and serum were assayed for virus. Serum antibody levels were also assessed.

With both viruses, there was little difference between antibody titres in pregnant mice compared to non-pregnant mice (Figures 3.4 and 3.5). Furthermore, following RRV infection, there was essentially no difference between the time course of virus growth in spleen, brain, liver and serum in pregnant mice compared to non-pregnant mice (Figure 3.4). In muscle, RRV titres were higher in pregnant mice early in infection and lower in pregnant mice after day 3 p.i. Following SFV infection, the virus titres in all tissues and in serum from pregnant mice were,

Table 3.5 The effect of pregnancy on the ability of SFV-immune spleen cells[a] to protect recipient mice against lethal SFV infection[b]

Dose of spleen cells per recipient	Percentage protected[c]	
	Pregnant spleen[d]	Non-pregnant spleen[e]
10^5	20	0
10^6	80	0
10^7	100	20
5×10^7	100	80

a Donor mice were infected i.p. with 7000 pfu of SFV 7 days prior to transfer.

b Recipient mice were challenged i.p. with 1000 pfu of virulent SFV 24 hours prior to i.v. administration of immune cells.

c 5 mice per group.

d Spleen cells from pregnant mice had a specific lysis of 50% at an effector:target ratio of 20:1.

e Spleen cells from non-pregnant mice had a specific lysis of 15% at an effector:target ratio of 20:1.

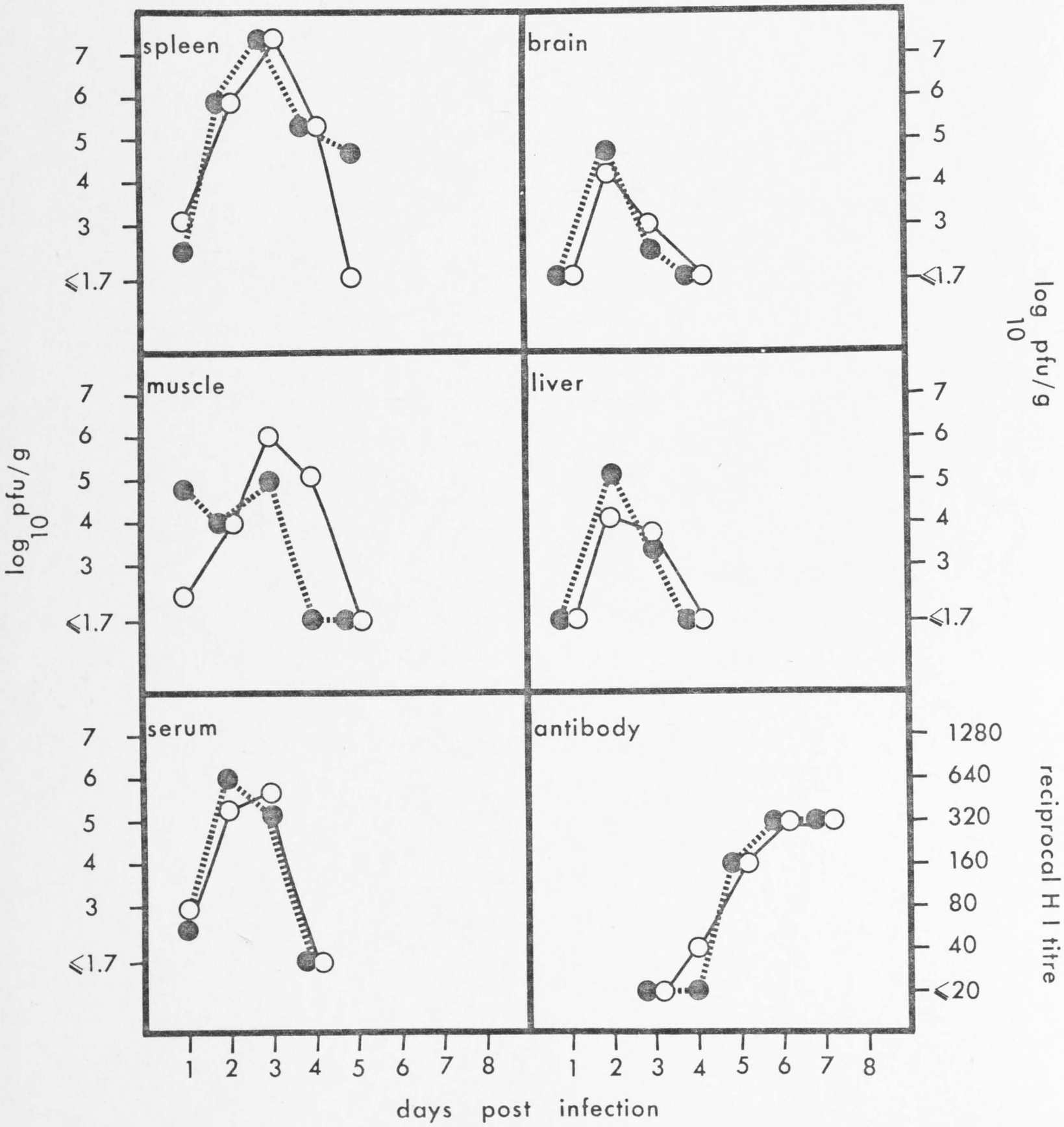
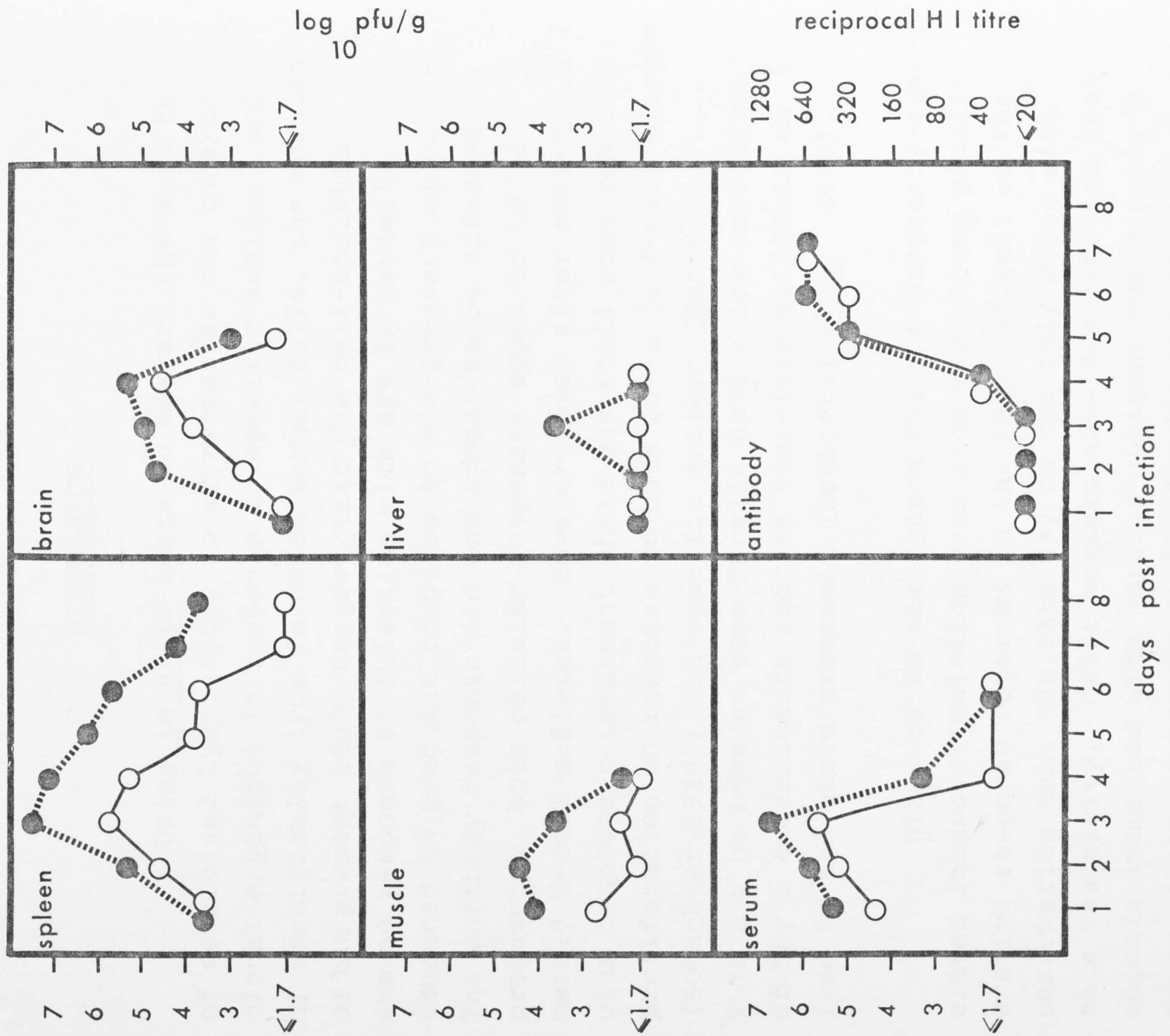

spleen
brain
muscle
liver
serum
antibody
7
6
5
4
3
≤1.7
1280
640
320
160
80
40
<20
1 2 3 4 5 6 7 8
days post infection
log10 pfu/g
reciprocal H I titre

Figure 3.5 Growth of SFV in the tissues of pregnant compared to non-pregnant mice.

Groups of 3 11-day pregnant mice and non-pregnant controls were infected i.p. with 7000 pfu of SFV. At different times after infection various tissues were pooled and assayed for virus. Serum antibody titres were also measured.

● = virus or antibody titre in pregnant mice

○ = virus or antibody titre in non-pregnant mice.


log pfu/g
10
7
6
5
4
3
≤1.7
brain
liver
spleen
muscle
log10 pfu/g
reciprocal H I titre
1280
640
320
160
80
40
≤20
antibody
serum
1 2 3 4 5 6 7 8
days post infection

unexpectedly, always equal to or higher than the corresponding titres in the non-pregnant mice (Figure 3.5). Nevertheless, the actual rate of clearance of SFV from the tissues of the pregnant mice appeared to be similar to that of the non-pregnants, suggesting that the immune mechanisms involved in the clearance of virus were operating at the same efficiency in both groups.

DISCUSSION

Using *in vitro* tests to measure the ability of sensitised lymphocytes to a) recognize and undergo clonal expansion in response to specific antigen, and b) specifically lyse infected target cells, the results of the present study suggest that the cell-mediated immune response to infection with SFV is markedly enhanced in pregnant compared to non-pregnant mice. The antibody response did not appear to be affected by pregnancy. Both cellular responses appeared to be mainly T-cell mediated. However, even after anti-Thy 1.2 plus complement treatment there was still some residual proliferation in response to both Con A (a T-cell mitogen (Schechter 1980)) and specific antigen (Table 3.3). This may be because some T-cells have a low expression of Thy 1 determinants and are therefore difficult to lyse by the above treatment (Ledbetter *et al*. 1980).

Hints as to the reason for the pregnancy-associated immuno-stimulation come from the strong proliferative response observed in the PALN, as well as the observation that the PALN may be the only nodes with this reactivity. Other workers have demonstrated that animals immunised with various antigens may display a strong immune response in the lymph nodes which drain the site of inoculation (Khoury *et al*. 1981; Snow and Hilgard 1981). This is because there is a specific selection of antigen-reactive cells from the recirculating lymphocyte pool into antigen-stimulated

lymph nodes (Hopkins et al. 1981) resulting in a more concentrated population of responsive cells (Griffin and Johnson 1973). This enhanced reactivity may also be observed in the spleen but only when a high antigen load is administered (Nash and Holle 1973). It may therefore be hypothesised that in the present study following infection with SFV the pregnant animal is hyper-immunised by a large antigenic load originating in the uterus and passing into the systemic circulation through the PALN. This is supported by the results of Chapter 4 which show that high concentrations of SFV are present in both placental and foetal tissue soon after maternal infection. An explanation for the small peak in ^{3}HTdR incorporation in control wells (i.e., those without added antigen) occurring at 6 days p.i. in the PALN of pregnant mice infected with SFV (Figure 3.2) may be that a proportion of the antigen draining into these nodes is retained by dendritic cells and causes the antigen-reactive cells to undergo blastogenesis (Nossal et al. 1968). Thus, their basal rate of label uptake would be higher than normal.

The results of Chapter 4 show that, as well as SFV, RRV also grows to high titre in foetal and placental tissue following maternal infection. Why then do we not see a markedly enhanced stimulation of the anti-RRV T-cell mediated immune responses in these mothers? One possible explanation is that the intra-uterine viral antigen is, under normal circumstances, relatively inaccessible to the mother's lymphatic system, and thus her immune response, but is made more available to the mother after the abortion always observed following infection with SFV at gestation days 10 or 11 but never with RRV (see Chapter 4). This hypothesis is based upon a number of experimental observations. It has been established by Barker and Billingham (1968) that intact lymphatic drainage from the site of allografting is an essential prerequisite for the sensitisation of the host to antigen but not the expression of the response against the antigen. In

the pregnant uterus, however, the decidual tissue which develops beneath the placenta and experimental homografts may interfere with maternal sensitisation against foreign intra-uterine antigens, although it does not affect the expression of transplantation immunity (Beer and Billingham 1971; Beer et al. 1971). This indicates that the decidual tissue blocks the entry of these antigens into the maternal lymphatic system. While this is not considered to play a major role in ensuring the success of the foetal allograft (Beer and Billingham 1971), it may delay or diminish the stimulation of immunity against virus within the pregnant uterus. The tissue disruption associated with SFV-induced abortion may bypass this apparent "lymphatic blockade" by the decidual tissue making viral antigen from the foeto-placental unit more available to the maternal immune system, perhaps by mechanisms analogous to those involved in maternal sensitisation against human foetal Rh antigens following a placental bleed at birth (Roitt 1974). The occurrence of abortion at 3½ to 5 days p.i. (Figure 4.6) would probably be in time to induce the immuno-stimulation which peaked at days 6 to 7 p.i. (Figures 3.2 and 3.3).

Using a similar hypothesis it could be argued that the availability of RRV antigen in infected foetal and placental tissue to the mother's lymphatic system may also have been increased following parturition, at days 8 to 9 p.i. (i.e., gestation days 19 to 20). However, this would have been too late to influence the initial stimulation of the immune response which was first detected in the PALN at day 6 p.i. (Figure 3.1).

Although the proliferative response to SFV antigen was higher in the pregnant PALN than in the spleen, the reverse was true with the cytotoxic response. The reason for this may be dependent on the observation that different T-cell subsets are involved in the 2 responses. Cantor and Boyse (1975) noted that peripheral mouse T-cells could be divided into 3 distinct sub-classes on the basis of their differential expression

of Ly-1, Ly-2 and Ly-3 surface antigens. Cells displaying Ly-2 and Ly-3 antigens (i.e., Ly-23$^+$) appeared to be cytotoxic whereas Ly-1$^+$ cells were helper cells for antibody production and had little cytotoxic activity. All cell groups proliferated in response to antigen. Thus, a different distribution of these T-cell subsets between the spleen and PALN in the present study would explain this phenomenon. An alternative explanation may be that the PALN in pregnant animals contains suppressor cells which non-specifically inhibit the generation of cytotoxic T-cells (Clark et al. 1980; Clark and McDermott 1981) but do not appear to greatly affect the proliferative responses to antigens or mitogens (Anderson 1978; Suzuki and Tomasi 1979).

Although the T-cell mediated immune response, as detected in vitro, was enhanced following SFV infection during pregnancy, virus titres in all organs examined tended to be higher in pregnant mice compared to non-pregnant mice (Figure 3.5). This appears paradoxical in the light of clinical and experimental observations which indicate that the generation of specific effector T-lymphocytes plays a critical role in recovery from primary virus infections (Woodruff and Woodruff 1975; Zinkernagel and Doherty 1979; Doherty and Bennink 1981). On their own, these results could be taken to indicate that the expression of anti-viral immunity is compromised during pregnancy. However, this would not be in accordance with the observations of Faulk and McIntyre (1981) and Siiteri and Stites (1982) who noted that pregnancy is associated with a relatively normal immune reactivity against tumours, skin grafts and infections. It would also be at variance with a number of other results from the present study. For instance, although Figure 3.5 indicates that titres of SFV are higher in the pregnant group, both the time of onset of the viral clearance mechanisms and the rate of viral clearance is similar in all tissues of the 2 groups. Furthermore, on a cell-to-cell basis, immune spleen cells from pregnant animals provide much greater protection against lethal infection with SFV than the same cells

from non-pregnant animals (Table 3.5). This is associated with enhanced cytotoxic activity and is probably not due to increased production of antibody by the pregnant spleen because the serum antibody titres appear to be unaffected by pregnancy (Figure 3.5). Finally, the differential between organ titres in pregnant animals compared to non-pregnant animals is not observed with RRV infection (Figure 3.4), suggesting that it is peculiar to the pathogenesis of SFV infection. It is tempting to speculate that the reason for these results, at least after the time of abortion, may again be linked to the previously proposed large stimulus of SFV antigen passing from the uterus to the mother's lymphatic system. In effect, this might mean that the pregnant animals were receiving a much higher and possibly more prolonged dose of infectious virus. Therefore, the SFV growth curves obtained in the 2 groups in Figure 3.5 may not be strictly comparable. Possibly a better measure of the relative extent of expression of anti-viral immunity in pregnant animals is obtained from Table 3.5 where the *in vivo* protective capacity of immune spleens from both groups of mice was assayed in recipients challenged with the *same* dose of virus.

In conclusion, this study has produced no evidence that the state of pregnancy *per se* is associated with a systemic depression of either the stimulation or expression of anti-viral immunity in the mother. On the contrary, when foetal or placental infection occurs, the intra-uterine viral antigen may even cause an enhancement of the mother's T-cell anti-viral responses. These results would not be predicted from those of other workers who detected numerous non-specific cellular and/or humoral immuno-suppressive mechanisms in pregnant subjects (Reviewed in Chapter 1.2). These phenomena were considered to be important for the continuation of an outbred pregnancy by suppressing the immunological rejection of the allogeneic foetus by the mother. As was noted in section 1.2.5, however, in a teleological sense, any explanation for the immunological "paradox" of

pregnancy which implicates mechanisms which would prejudice the ability of the mother to combat infection or neoplastic growth is extremely poor, and does not fit in with the observations of maternal immuno-competence during gestation. Nevertheless, the results of this Chapter do not rule out the possibility that immuno-suppressive mechanisms may operate in the micro-environment of the placenta to prevent maternal reactivity against trophoblast. If this is the case, immune responses against virus-infected trophoblast may also be compromised.

CHAPTER 4

THE EFFECT ON THE PREGNANCY OF MATERNAL INFECTION WITH ROSS RIVER VIRUS OR SEMLIKI FOREST VIRUS

INTRODUCTION

In Chapter 3 it was established that both RRV and SFV replicate in the maternal tissues of the pregnant mouse without causing any apparent ill-effects in the mother. Furthermore, these infections were limited, in both cases recovery being associated with the formation of cell-mediated and humoral immune responses. It was suggested that the strong anti-viral activity detected in the PALN following SFV infection and, to a lesser extent, RRV infection, resulted from a large viral antigen stimulus originating in the uterus and moving into the mother via her lymphatic system. This Chapter therefore concentrates on the foeto-placental unit and how it is affected by maternal infection with these viruses.

As was noted in Chapter 1, the majority of maternal infections during pregnancy are inconsequential for the foetus. Nevertheless, some viruses are able to adversely affect foetal development because of changes in the health and continued well-being of the mother or placenta, or by direct infection of the foetus itself. The factors which determine whether or not a foetus will be infected following maternal virus disease are diverse and include virus virulence and tropisms, previous maternal exposure to virus or vaccine, immuno-competence of the foetus, stage of gestation and, possibly, host genotype. The placenta may also play a role as a "barrier" against trans-placental passage of the virus.

The experiments described in this Chapter examine the time-course of events in the placenta and foetus following maternal infection with RRV or SFV. It was hoped that this would provide an understanding of aspects of the pathogenesis of the resulting foetal diseases, with particular reference to the mechanisms of virus entry and growth in placental and foetal tissue; the consequences of foetal infection; the effect of the onset of the maternal immune response

on the course of the pregnancy; and the role, if any, of the placenta as a barrier to infection.

RESULTS

Time course of events following maternal infection with RRV.

Groups of 4 or 5 11-day pregnant mice were infected i.p. with 2600 pfu of RRV, and killed at various times after, up to day 7 p.i. Serum, placentas and foetuses were taken for virus assay. Viraemia was detected in most mice at day 1 p.i., all mice at day 1.5 p.i. and peaked at day 2 p.i. (Figure 4.1). Virus was not detected at day 3 p.i. The rise in serum antibody titre inversely reflected the decline in the viraemia (Figure 4.1).

Time course of growth of RRV in placental tissue

The time of appearance and concentration of RRV was assayed in the individual placentas from these mothers (Figure 4.2). Virus was first detected in a proportion of the placentas harvested 1 day after maternal infection, corresponding to the time of onset of the maternal viraemia (Figure 4.1). Placental titres rose rapidly until days 2 to 3 p.i., and were still high at the end of the experiment at day 7 p.i. After day 1 p.i., all placentas contained virus, and by day 2 p.i., the level of virus in most placentas exceeded that in blood. There was no difference between the placental virus titres from dead foetuses compared to living foetuses. Abortion was not observed.

Time course of growth of RRV in foetal tissue

The foetuses from these mothers were individually assayed for virus (Figure 4.3). RRV was

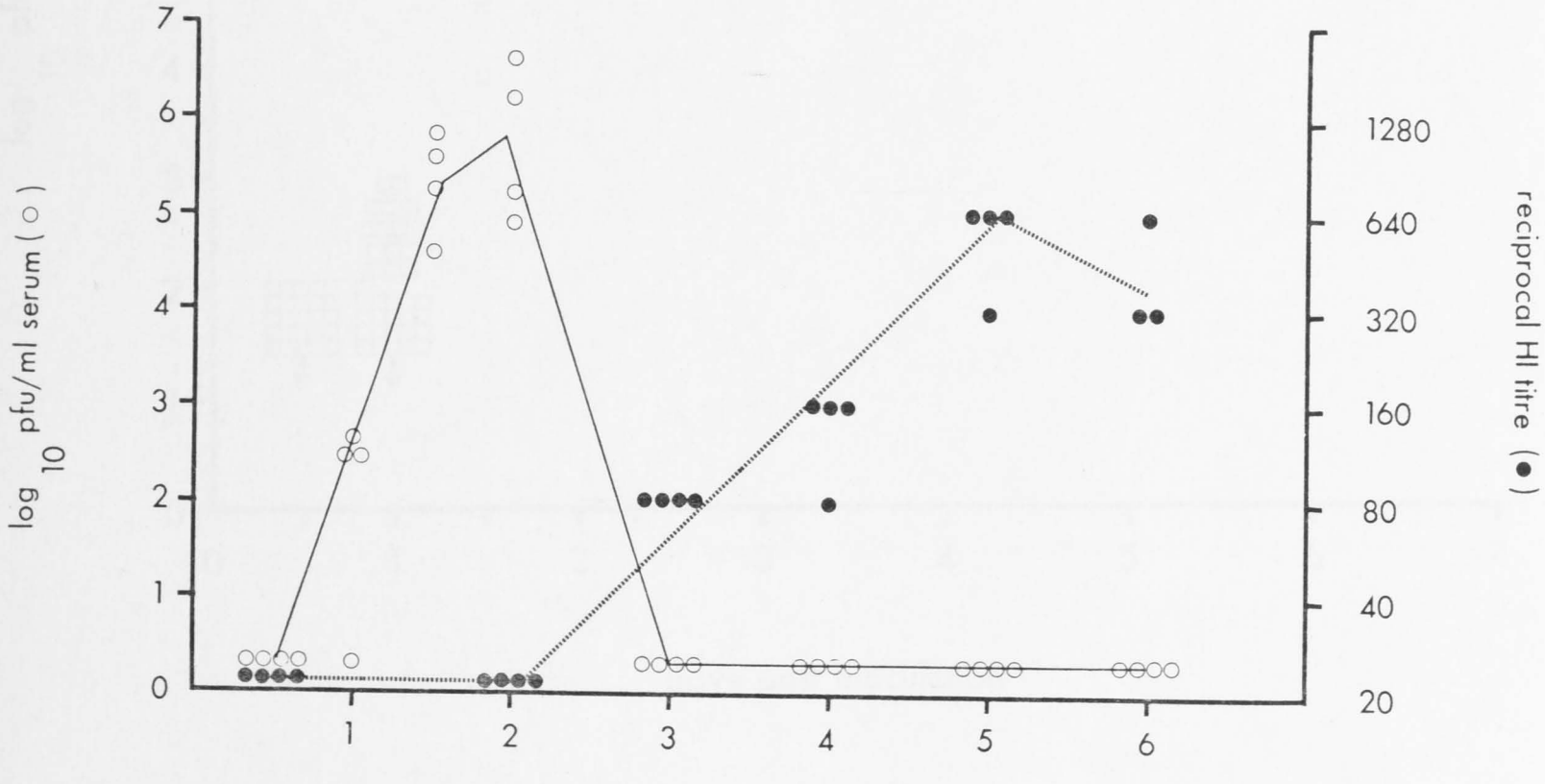


Figure 4.1 Time course of viraemia and antibody production in pregnant mice infected with RRV.

Groups of 11-day pregnant mice were infected i.p. with 2600 pfu of RRV. Individual serum samples were assayed for virus and antibody at the times shown.

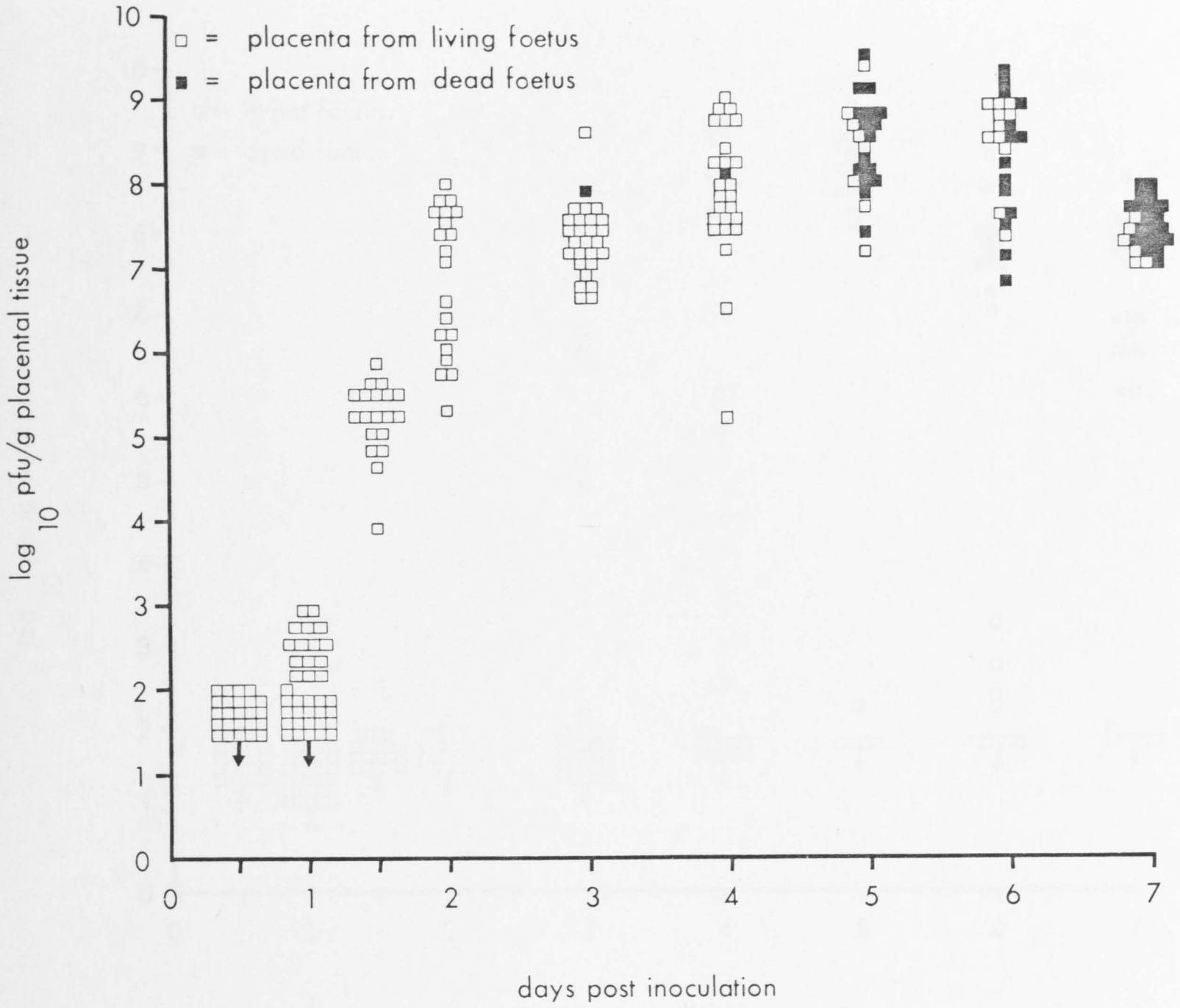


Figure 4.2 Time course of growth of RRV in placental tissue.

Groups of 11-day pregnant mice were infected i.p. with 2600 pfu of RRV. Individual placentas were assayed for virus content at the times shown.

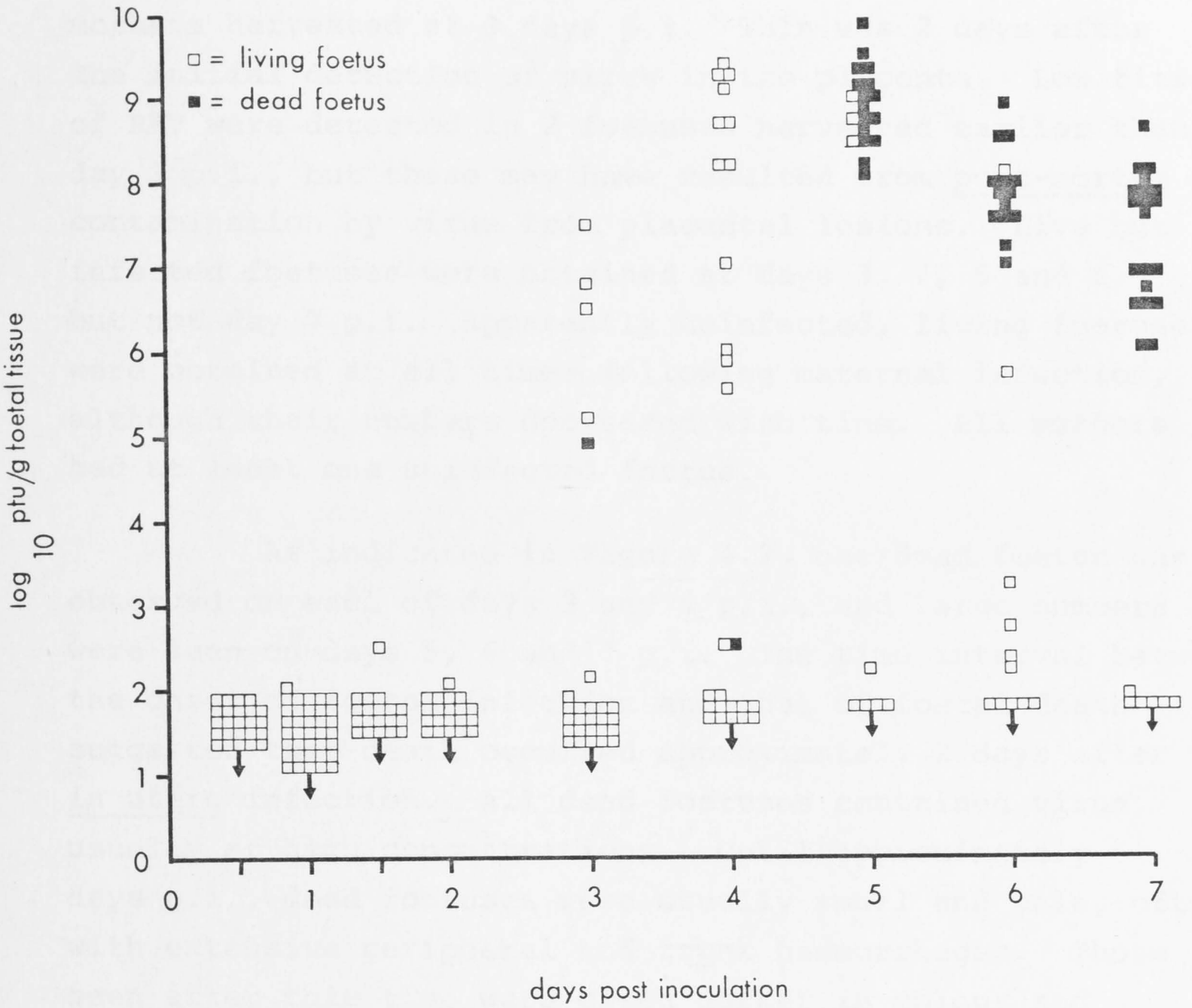


Figure 4.3 Time course of growth of RRV in foetal tissue.

Groups of 11-day pregnant mice were infected i.p. with 2600 pfu of RRV. Individual foetuses were assayed for virus content at the times shown.

first detected at high titre in some foetuses from the mothers harvested at 3 days p.i. This was 2 days after the initial detection of virus in the placenta. Low titres of RRV were detected in 2 foetuses harvested earlier than day 3 p.i., but these may have resulted from post-mortem contamination by virus from placental lesions. Live but infected foetuses were obtained at days 3, 4, 5 and 6, but not day 7 p.i. Apparently uninfected, living foetuses were obtained at all times following maternal infection, although their numbers decreased with time. All mothers had at least one uninfected foetus.

As indicated in Figure 4.2, one dead foetus was observed on each of days 3 and 4 p.i., and large numbers were seen on days 5, 6 and 7 p.i. The time interval between the onset of foetal infection and that of foetal death suggested that death occurred approximately 2 days after in utero infection. All dead foetuses contained virus, usually at high concentrations. Until approximately 6 days p.i., dead foetuses were usually small and pale, often with extensive peripheral and trunk haemorrhages. Those seen after this time were often darker in colour and mummified. No gross anatomical abnormalities were seen. There was no significant difference between the weight of live foetuses from infected mothers at gestation day 18 (mean $\pm$ S.D. = 1.01 $\pm$ 0.10 g; n = 23) to those from uninfected mothers at the same stage of gestation (mean $\pm$ S.D. = 1.01 $\pm$ 0.09 g; n = 66). A typical pregnancy 6 days after maternal infection with RRV is presented in Figure 4.4.

Time course of events following maternal infection with SFV

A similar time course experiment was performed with SFV. Groups of 11-day pregnant mice were infected i.p. with 7000 pfu of SFV and harvested at different times after inoculation. Individual sera from 4 mice at each harvest time were assayed for virus and HI antibodies (Figure 4.5). Viraemia was first detected at 1 to 1½ days p.i., peaked at 3 days p.i., and was

Figure 4.4 A typical pregnancy 6 days after i.p. infection of an 11-day pregnant mouse with 2600 pfu of RRV. The uterine wall is opened exposing the foetuses within their membranes. Two dead foetuses (arrowed) and 4 living foetuses are present. Note the dead foetus on the right has a distal tail haemorrhage.

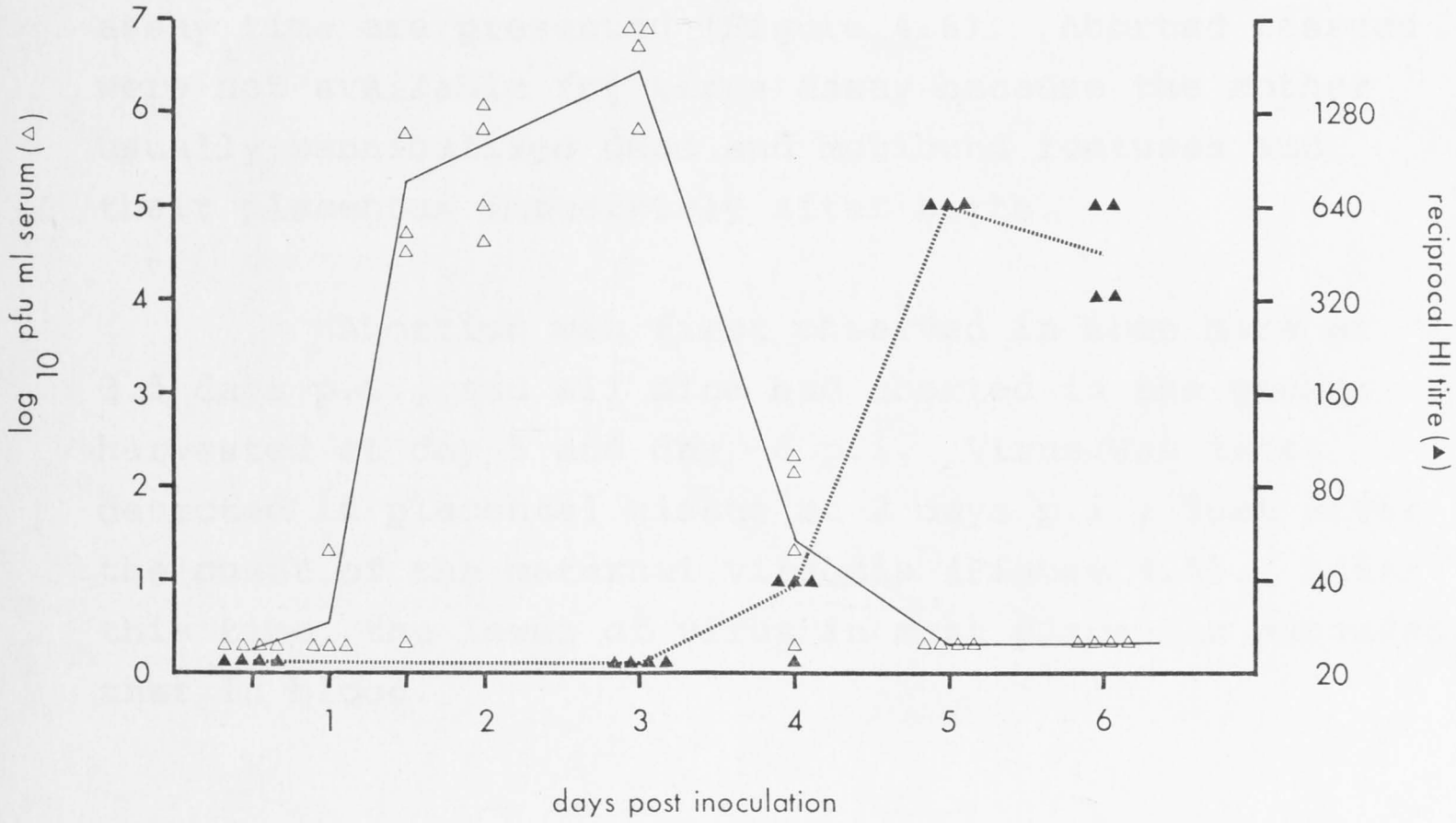


Figure 4.5 Time course of viraemia and antibody production in pregnant mice infected with SFV.

Groups of 11-day pregnant mice were infected i.p. with 7000 pfu of SFV. Individual serum samples were assayed for virus and antibody at the times shown.

undetectable in all animals at 5 days p.i. The rise in serum antibody inversely reflected the decline in viraemia.

Time course of growth of SFV in placental tissue

Because infection with SFV caused abortion in some animals, both the individual placental virus titres and the proportion of infected mice that aborted at each assay time are presented (Figure 4.6). Aborted tissues were not available for virus assay because the mother usually cannibalized dead and moribund foetuses and their placentas immediately after birth.

Abortion was first observed in some mice at 3.5 days p.i., and all mice had aborted in the groups harvested at day 5 and day 6 p.i. Virus was first detected in placental tissue at 2 days p.i., just after the onset of the maternal viraemia (Figure 4.5). After this time, the level of virus in most placentas exceeded that in blood.

Time course of growth of SFV in foetal tissue

The amount of virus in individual foetuses was determined (Figure 4.7). Because of abortion, few dead foetuses were observed following SFV infection of pregnant mice, although all had high virus titres. Dead foetuses were usually pale, with extensive peripheral and trunk haemorrhages. No anatomical abnormalities were observed. Foetal infection was first observed in a group of mothers harvested at 3 days p.i. This was 1 day after the initial detection of virus in placental tissue, although the time of onset of foetal infection did not appear to be uniform because both mothers harvested at 4 days p.i. had uninfected foetuses. Where foetal infection occurred, it tended to be detected in all foetuses within a particular pregnancy, i.e., an "all

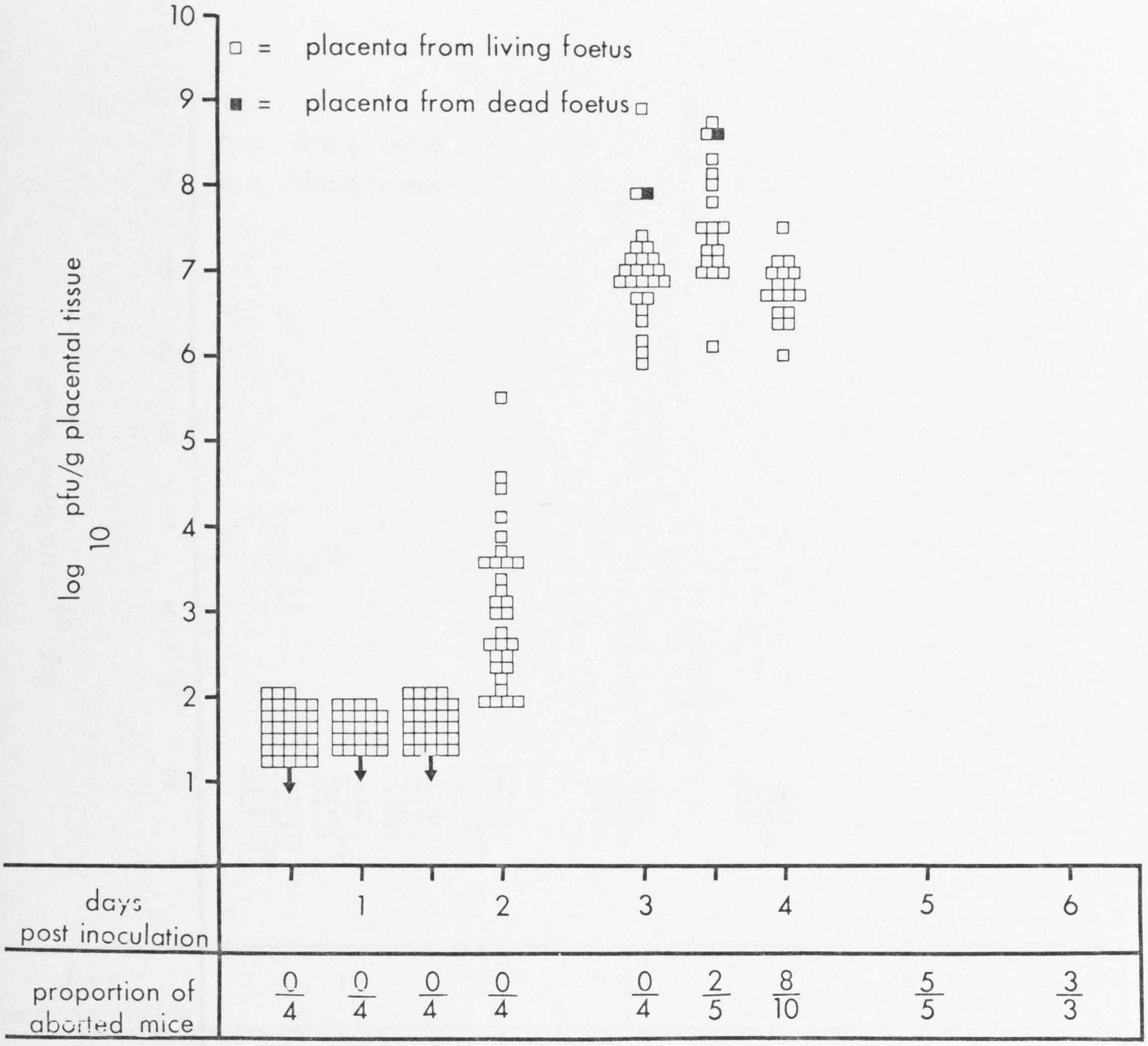
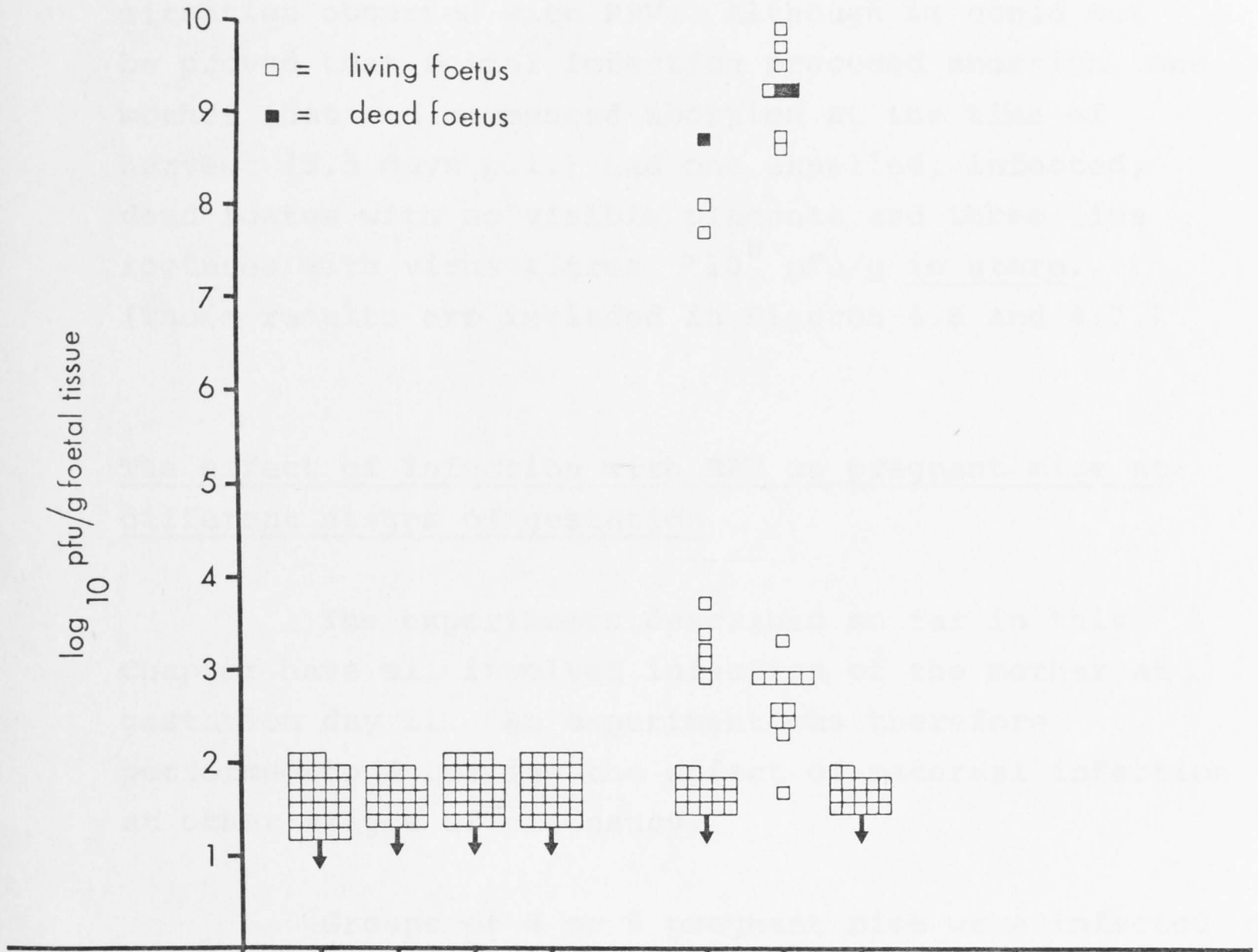


Figure 4.6 Time course of growth of SFV in placental tissue.

Groups of 11-day pregnant mice were infected i.p. with 7000 pfu of SFV. Individual placentas were assayed for virus content at the times shown. The proportion of mice that had aborted at each harvest time is also indicated.



days post inoculation	1		2		3		4		5		6	
proportion of aborted mice	0/4	0/4	0/4	0/4	0/4	2/5	8/10		5/5		3/3	

Figure 4.7 Time course of growth of SFV in foetal tissue.

Groups of 11-day pregnant mice were infected i.p. with 7000 pfu of SFV. Individual foetuses were assayed for virus content at the times shown. The proportion of mice that had aborted at each harvest time is also indicated.

or nothing" situation. This contrasts with the situation observed with RRV. Although it could not be proved that foetal infection preceded abortion, one mother that had commenced abortion at the time of harvest (3.5 days p.i.) had one expelled, infected, dead foetus with no visible placenta and three live foetuses with virus titres $>10^8$ pfu/g *in utero*. (These results are included in Figures 4.6 and 4.7.)

The effect of infection with RRV on pregnant mice at different stages of gestation

The experiments described so far in this Chapter have all involved infection of the mother at gestation day 11. An experiment was therefore performed to determine the effect of maternal infection at other stages of pregnancy.

Groups of 4 or 5 pregnant mice were infected i.p. with 2600 pfu of RRV at day 5, 7, 10, 13 or 16 of pregnancy. All mothers were harvested at gestation day 18 and individual foetuses and placentas were assayed for their virus content. Abortion was not observed at any time. Virus was detected in all but 15 placentas obtained from mothers infected at either gestation day 5 or day 7 (Figure 4.8). An extremely wide variation in placental virus titres was observed in the mothers infected at gestation day 5.

Uninfected, living foetuses were observed in all groups (Figure 4.9). With only one exception (a mother infected at gestation day 10), all mothers had at least one living, uninfected foetus. Infected, living foetuses were mainly observed in the group infected at gestation day 13, suggesting that this was the predominant group where active foetal infection was occurring at the time of harvest. It is presumed that there was insufficient time for foetal infection to occur in the group infected at gestation day 16.

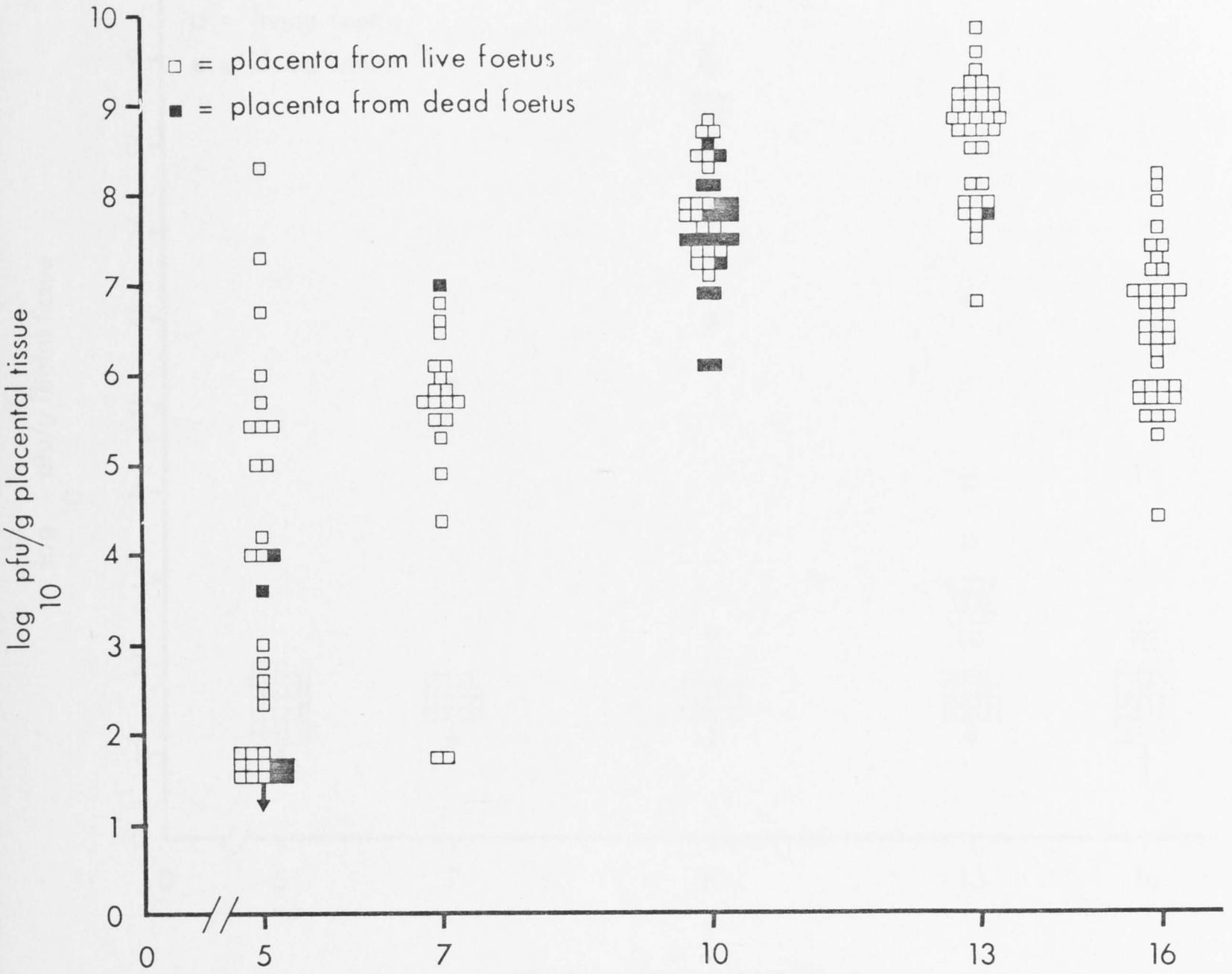


Figure 4.8 Effect of infection of mice with RRV at different stages of gestation: Placental virus titres.

Groups of 4 or 5 mice were infected i.p. with 2600 pfu of RRV at day 5, 7, 10, 13 or 16 of gestation. All groups were harvested at gestation day 18. Virus titres in individual placentas are plotted against day of inoculation.

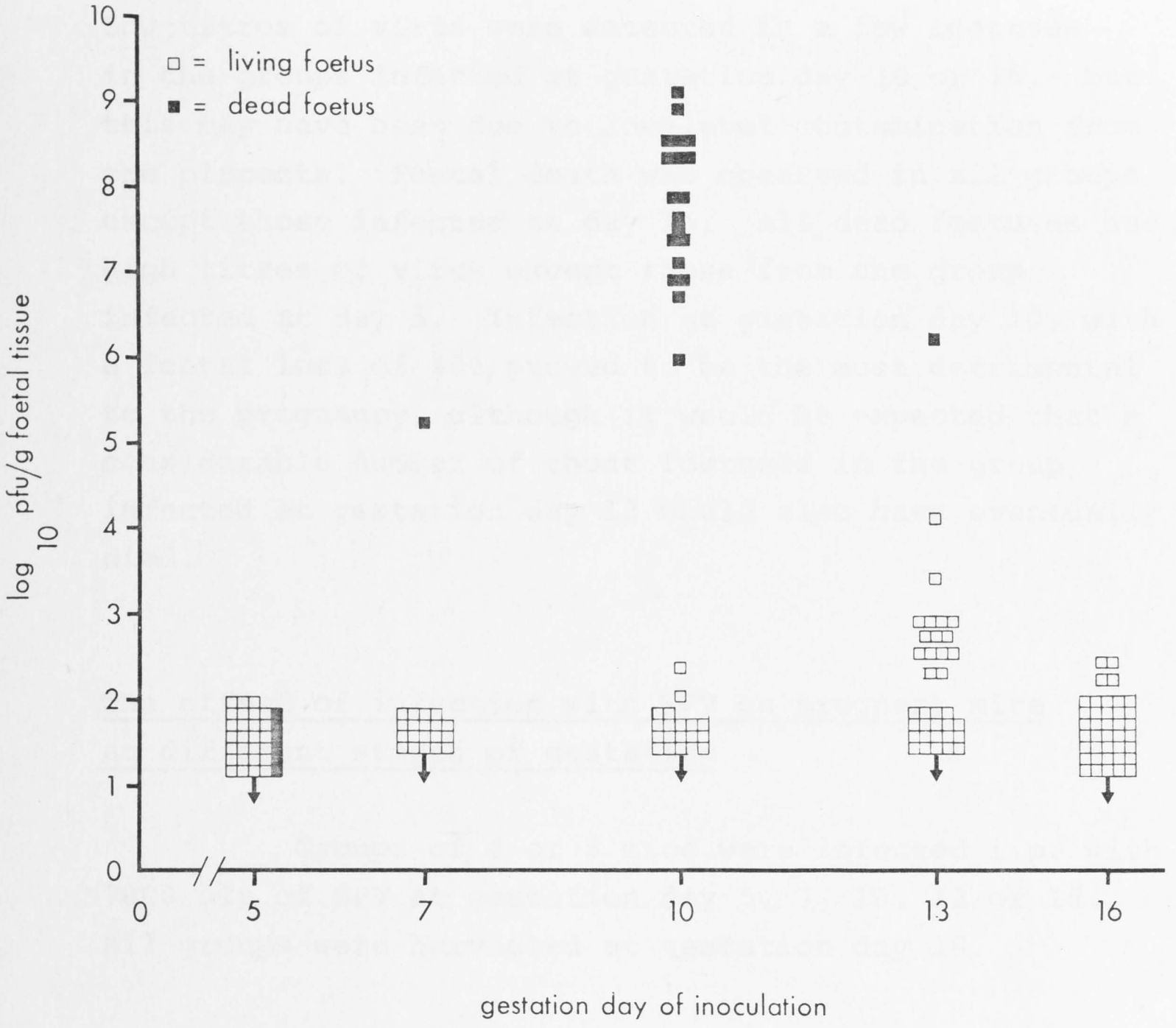


Figure 4.9 Effect of infection of mice with RRV at different stages of gestation: Foetal virus titres.

Groups of 4 or 5 mice were infected i.p. with 2600 pfu of RRV at day 5, 7, 10, 13 or 16 of gestation. All groups were harvested at gestation day 18. Virus titres in individual foetuses are plotted against day of inoculation.

Low titres of virus were detected in a few foetuses in the groups infected at gestation day 10 or 16, but this may have been due to low-level contamination from the placenta. Foetal death was observed in all groups except those infected at day 16. All dead foetuses had high titres of virus except those from the group infected at day 5. Infection at gestation day 10, with a foetal loss of 40%, proved to be the most detrimental to the pregnancy, although it would be expected that a considerable number of those foetuses in the group infected at gestation day 13 would also have eventually died.

The effect of infection with SFV on pregnant mice at different stages of gestation

Groups of 4 or 5 mice were infected i.p. with 7000 pfu of SFV at gestation day 5, 7, 10, 13 or 16. All groups were harvested at gestation day 18.

Abortion was not observed in groups infected at gestation day 5 or 16, but was observed in some animals infected at gestation day 7 or 13, and in all mice infected at gestation day 10 (Figure 4.10). There may have been insufficient time for abortion to occur in the one mouse remaining in the day 13 group, and in all the mice infected at gestation day 16. Of the mice that did not abort, the only placentas without virus were 9 from mice infected at gestation day 5. Some foetal death and resorption was observed in the mice infected at gestation day 5 or 7, although not all dead foetuses contained virus (Figure 4.11). Dead foetuses were not observed in the mice that had not aborted after infection at gestation day 13 or 16. All mothers that had not aborted at the time of harvest had at least one apparently uninfected foetus.

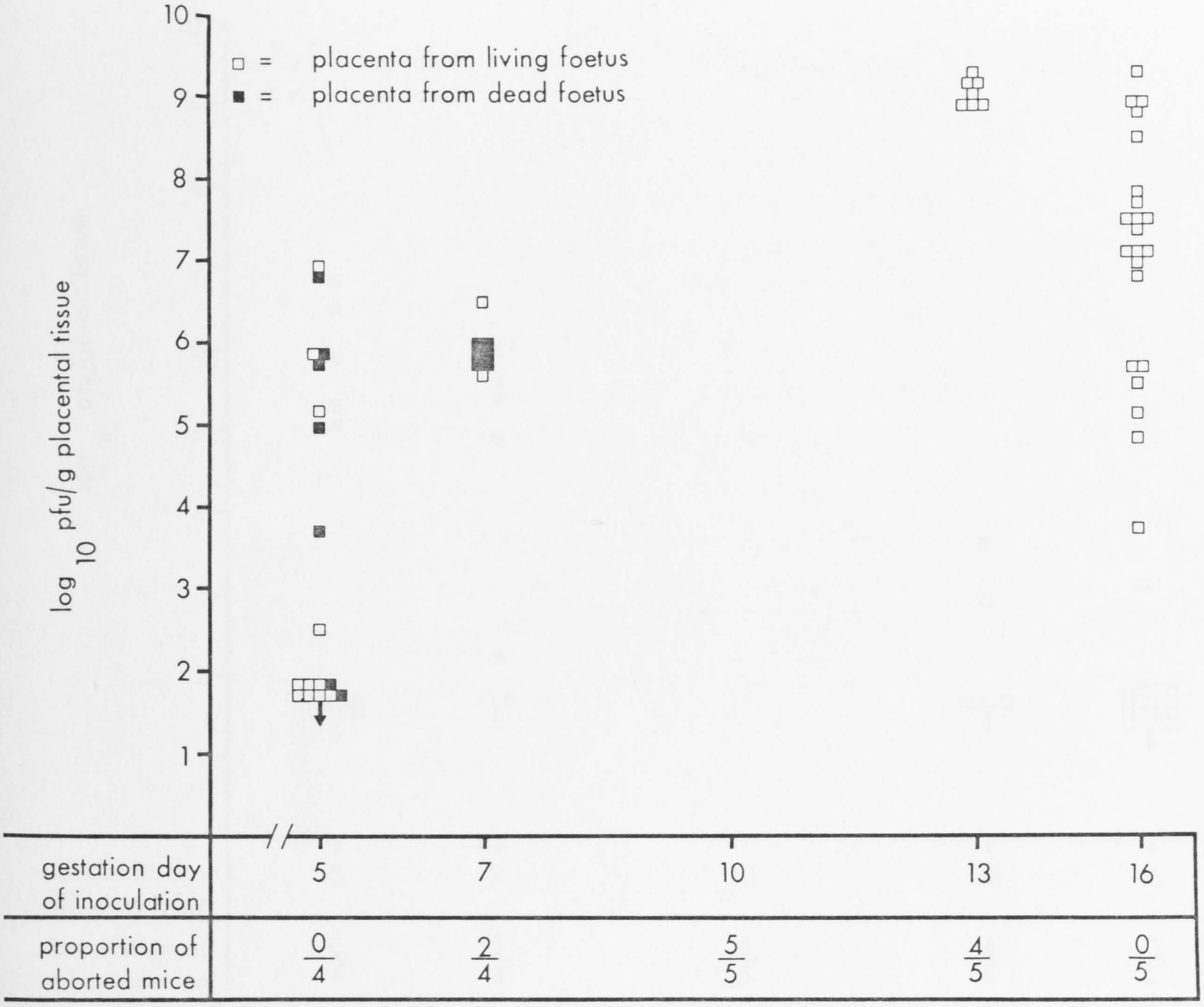


Figure 4.10 Effect of infection of mice with SFV at different stages of gestation: Placental virus titres.

Groups of 4 or 5 mice were infected i.p. with 7000 pfu of SFV at day 5, 7, 10, 13 or 16 of gestation. All groups were harvested at gestation day 18. Virus titres in individual placentas are plotted against day of inoculation. The proportion of mice that had aborted in each group is also indicated.

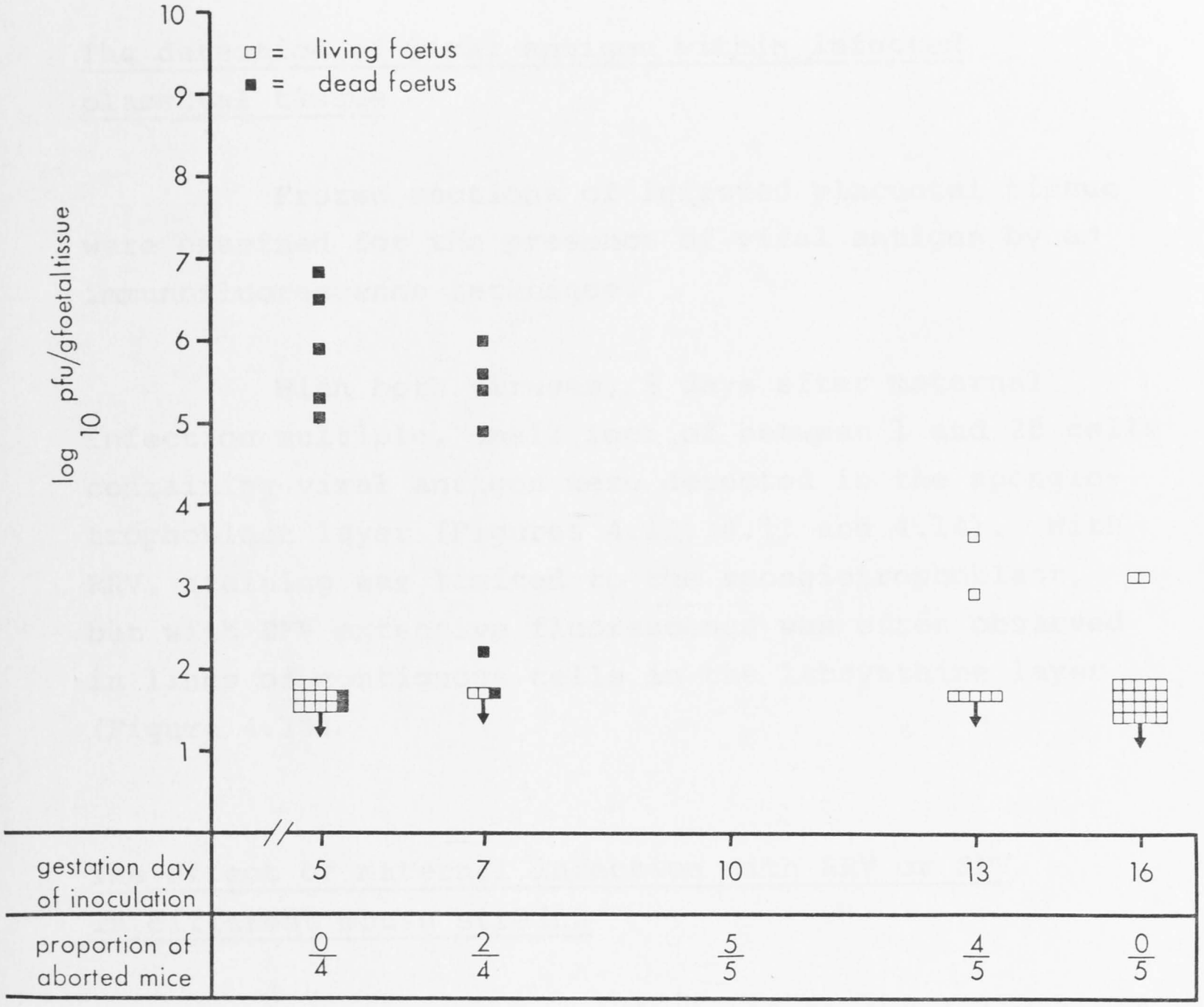


Figure 4.11 Effect of infection of mice with SFV at different stages of gestation: Foetal virus titres.

Groups of 4 or 5 mice were infected i.p. with 7000 pfu of SFV at day 5, 7, 10, 13 or 16 of gestation. All groups were harvested at gestation day 18. Virus titres in individual placentas are plotted against day of inoculation. The proportion of mice that had aborted in each group is also indicated.

The detection of viral antigen within infected placental tissue

Frozen sections of infected placental tissue were examined for the presence of viral antigen by an immunofluorescence technique.

With both viruses, 5 days after maternal infection multiple, small foci of between 1 and 20 cells containing viral antigen were detected in the spongiotrophoblast layer (Figures 4.12, 4.13 and 4.14). With RRV, staining was limited to the spongiotrophoblast, but with SFV extensive fluorescence was often observed in lines of contiguous cells in the labrynthine layer (Figure 4.13).

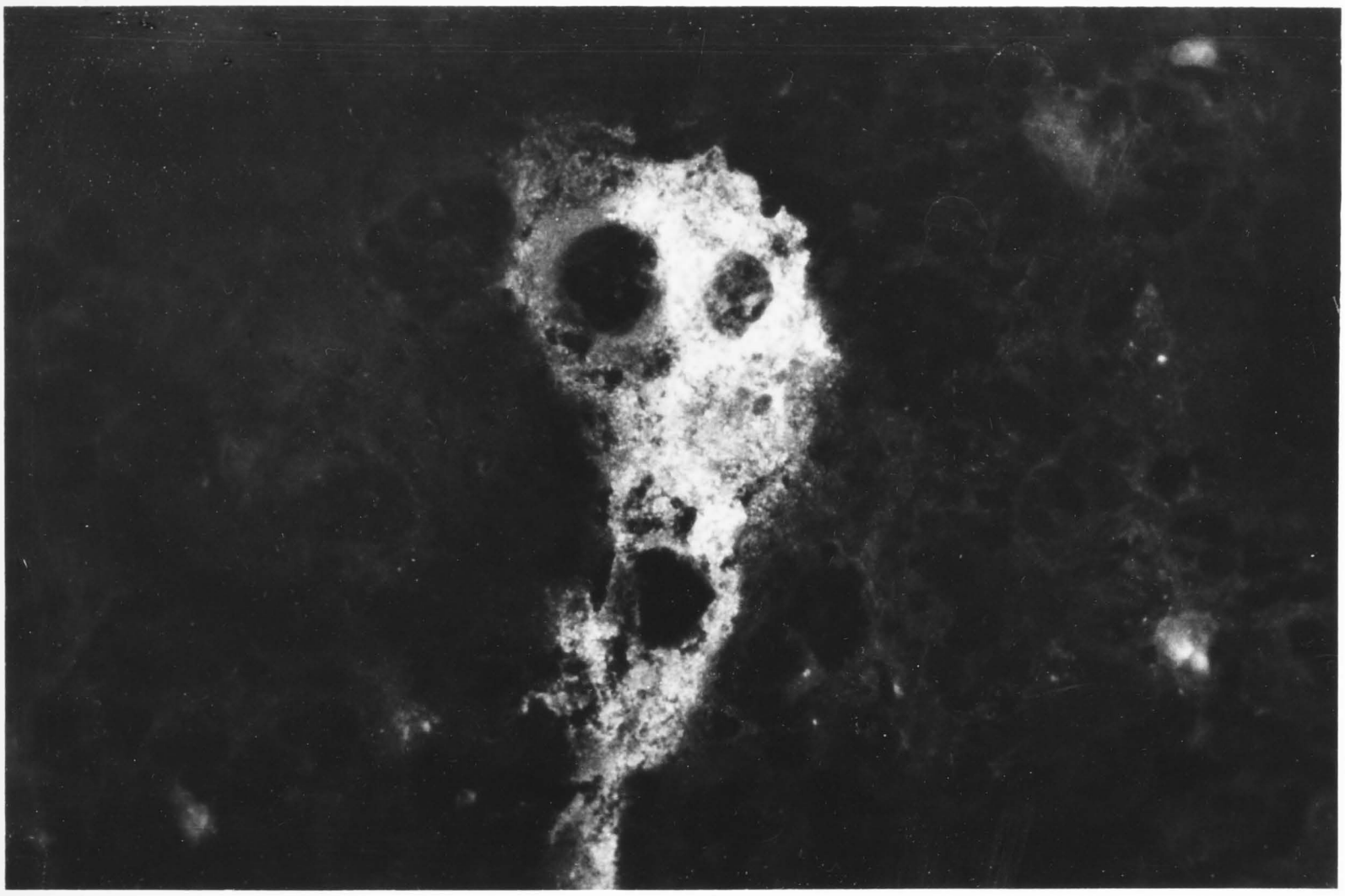
The effect of maternal infection with RRV or SFV in different mouse strains

An experiment was designed to examine whether the occurrence of *in utero* infection with RRV or SFV was linked to the presence of the CBA/H strain genotype or, alternatively, whether placental and foetal infection could be expected to occur in all mouse strains irrespective of their genetic make-up.

Groups of 4 pregnant mice from each of 5 commonly used mouse strains were infected i.p. with either 2600 pfu of RRV or 7000 pfu of SFV at gestation day 10. Foetuses and placentas were removed at 4 days p.i. and pooled for virus titrations. The results are summarised in Table 4.1.

Virus was detected at similar levels to that described previously in all placental pools in each strain for each infection. Virus was also detected in most foetal pools. In the 4 days between infection and harvest, abortion was not observed following RRV infection, but was seen in 3 of the 5 strains infected

Figure 4.12 Immunofluorescence: foci of viral antigen in spongiotrophoblast 5 days after maternal i.p. infection with 2600 pfu of RRV (x 132).



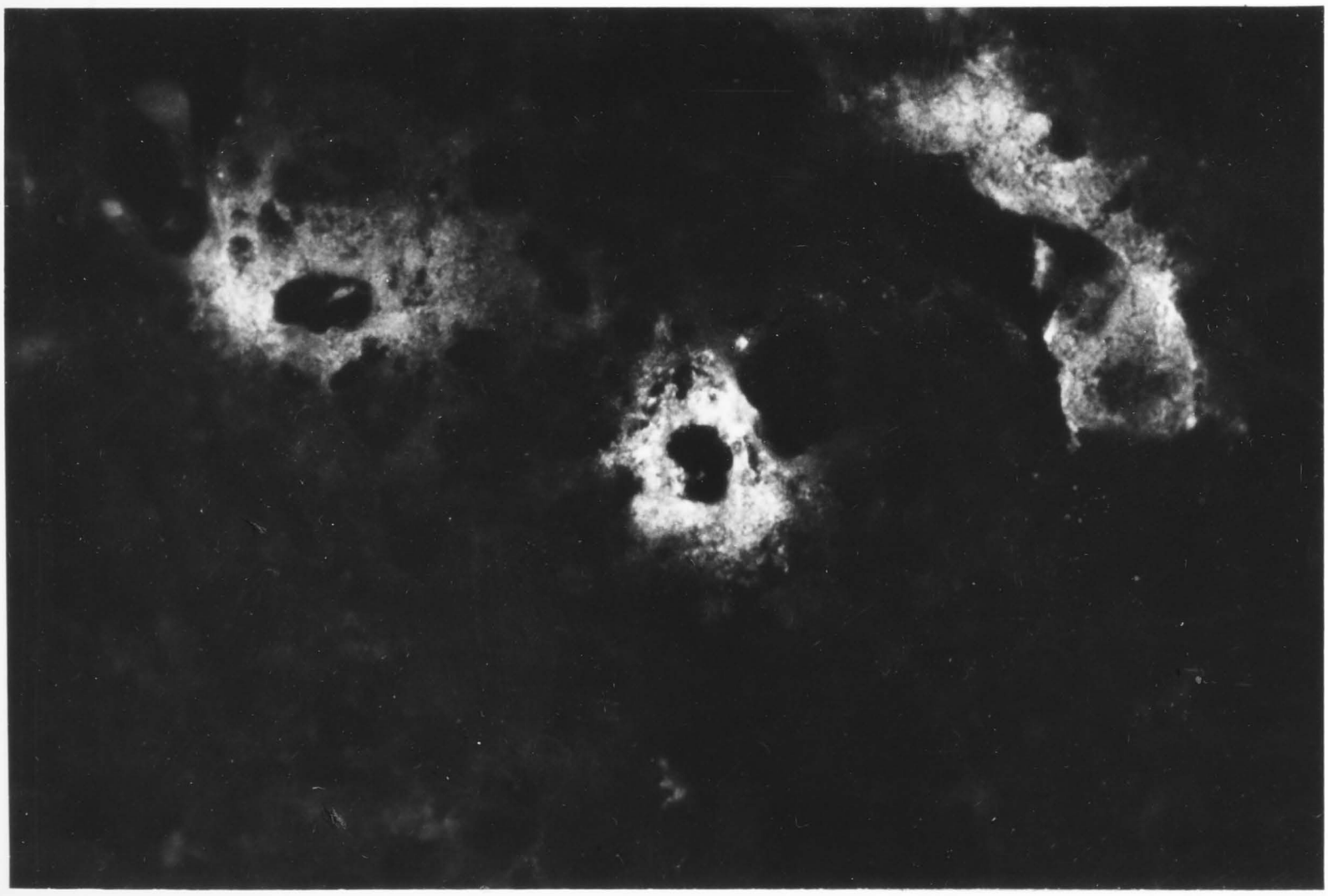
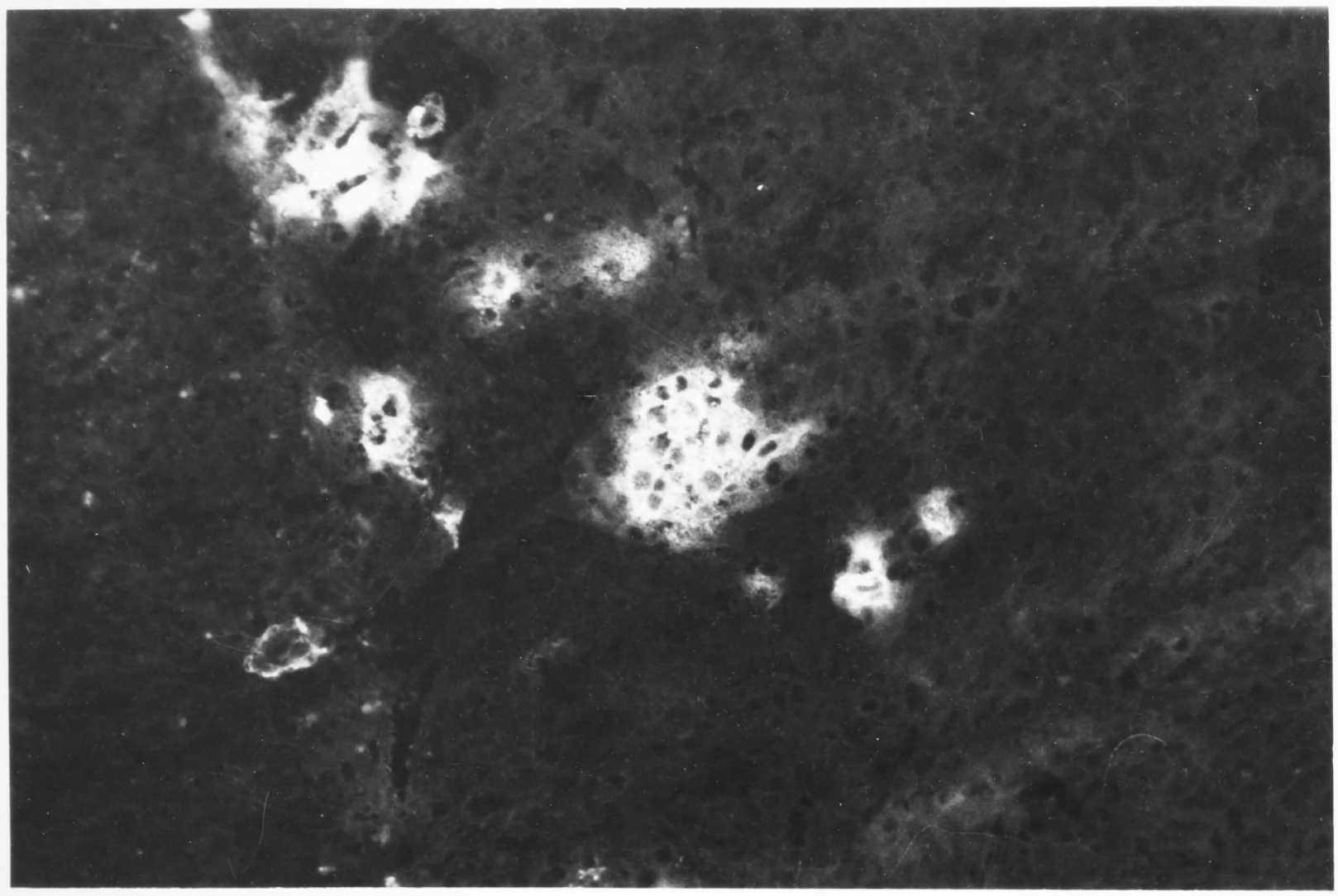


Figure 4.13 Immunofluorescence: foci of viral antigen in spongiotrophoblast 5 days after maternal i.p. infection with 2600 pfu of RRV (Top), and in the labrynthine layer after maternal i.p. infection with 7000 pfu of SFV (Bottom). (Both x 33)



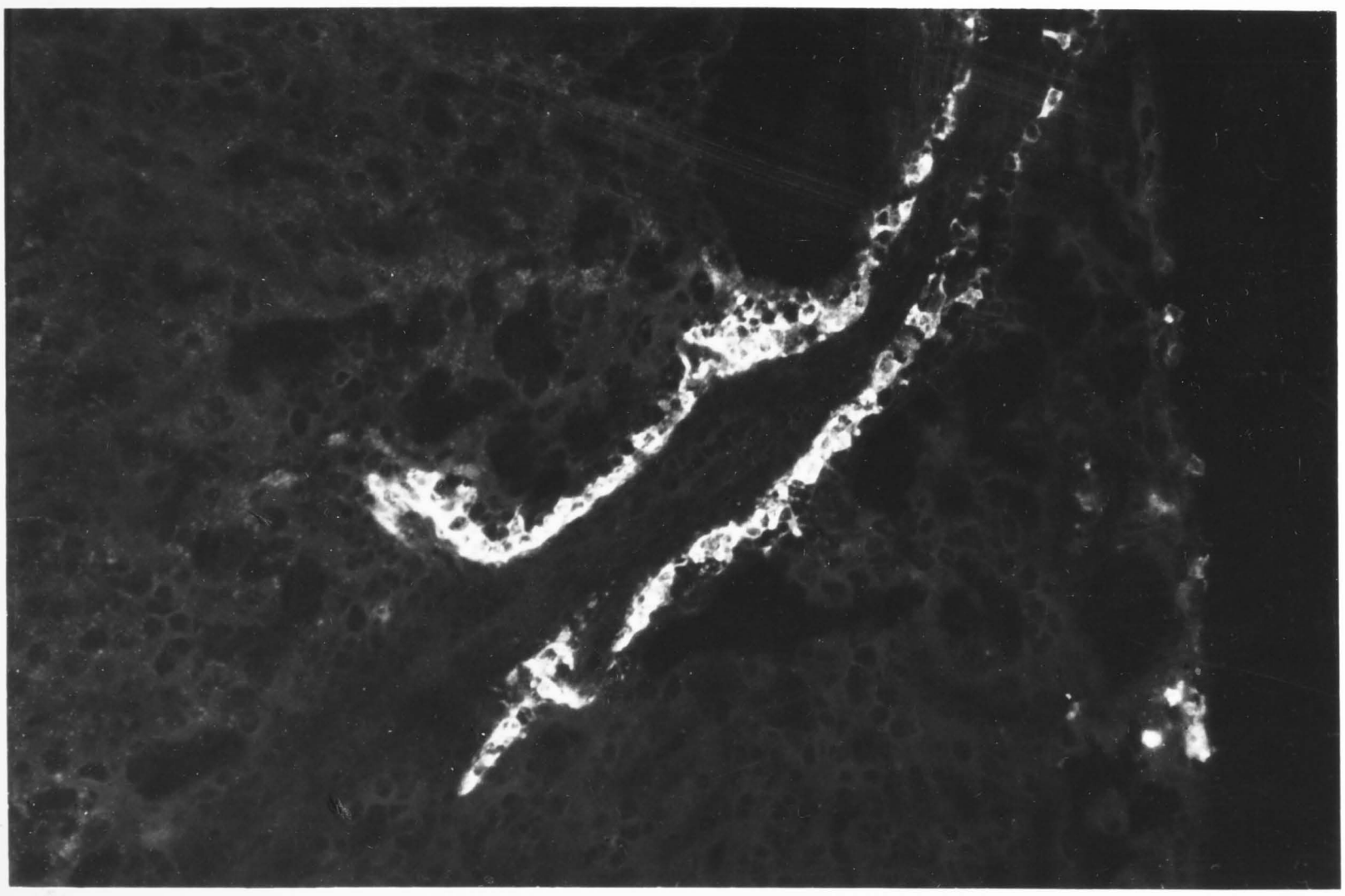
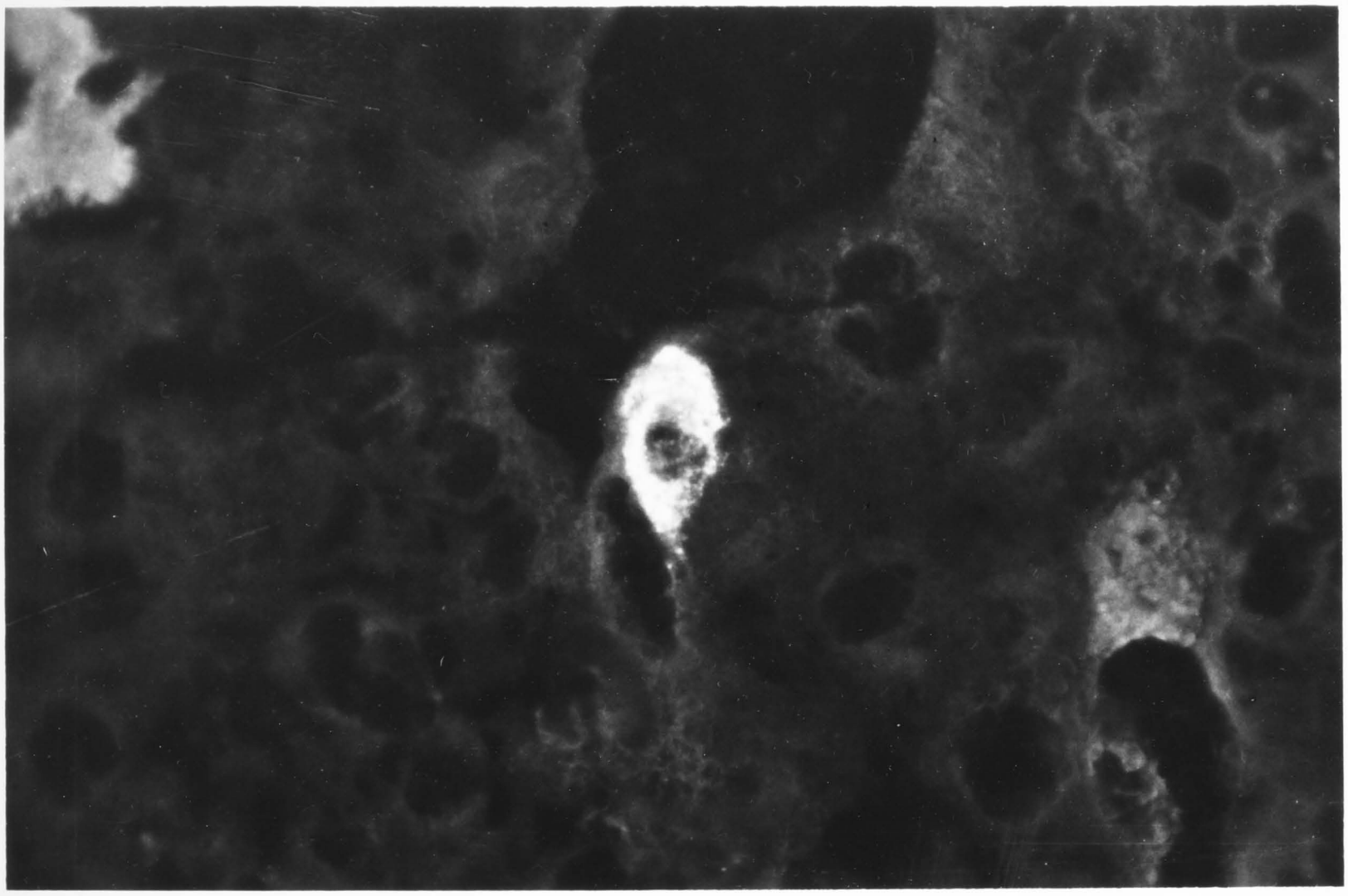


Figure 4.14 Immunofluorescence: foci of viral antigen in spongiotrophoblast 5 days after maternal i.p. infection with 7000 pfu of SFV. (x 132)



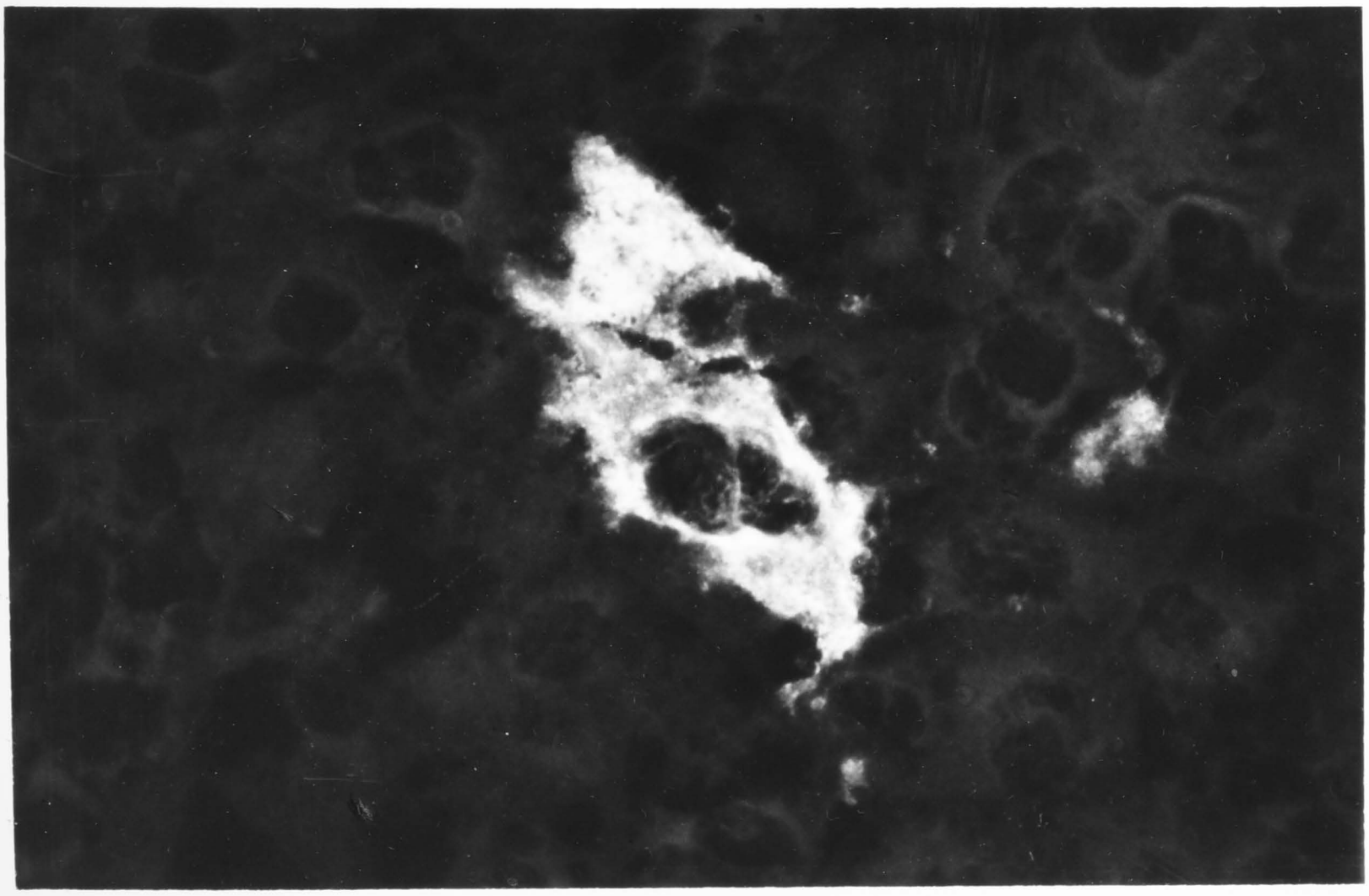


Table 4.1 The effect of infection[a] with RRV or SFV during pregnancy in different mouse strains

Strain	haplotype	RRV			SFV		
		Abortion	Foetal Infection	Placental Infection	Abortion	Foetal Infection	Placental Infection
CBA/H	k	-[b]	+[c]	+	+	+	+
Balb/c	d	-	+	+	-	+	+
A/J	a	-	+	+	+	+	+
DBA/1	q	-	+	+	-	+	+
NZB	d	-	+	+	+	+	+

a Groups of 4 pregnant mice were infected i.p. with either 2600 pfu of RRV or 7000 pfu of SFV at gestation day 10.

b - signifies not observed to occur.

c - signifies observed.

with SFV. If the time between infection and harvest had been extended beyond 4 days it is possible that SFV may have induced abortion in all of the infected strains.

These results suggest that, following infection of pregnant mice with either RRV or SFV, placental and foetal infection occurs irrespective of the maternal genetic make-up.

The role of the maternal immune response in the outcome of *in utero* infection with SFV

The role of the maternal anti-viral immune response in the pathogenesis of *in utero* infection with SFV was examined. Initial investigation concentrated on the coincidence between the onset of detectable T-cell anti-viral activity in the PALN and the occurrence of abortion. Both events are first detected at 3.5 to 4 days p.i. (Figures 3.2 and 4.6). Thus, the possibility that the abortion caused by SFV may have been triggered by a T-cell mediated immuno-pathological process was investigated by observing the effect of an ATS-induced T-cell depletion on the course of the infection.

Groups of pregnant mice were given 0.1 ml s.c. of either ATS or NRS at gestation days 9, 10, 11, 12, 13, 14 and 15, and were infected i.p. with 7000 pfu of SFV at gestation day 11. The days that abortion occurred and the antibody titres of the pooled maternal sera are presented in Table 4.2. The proliferative responses to Concanavalin A and SFV antigen in individual PALN and spleens from both groups were assessed in the LST at day 7 p.i. (Table 4.3). Other pregnant mice were treated with ATS or NRS and infected in the same way but were killed 3 days after infection to examine the effect of T-cell depletion on the titres of virus in the foetus, placenta and maternal serum (Table 4.4).

Table 4.2 The effect of anti-thymocyte serum[a] on the time of abortion and on maternal serum antibody titre following infection[b] with SFV

Group	Time of abortion (Days after infection)	Maternal serum HI antibody titre
ATS[c]	4; 4; 4; 4; 5	1:80
NRS[d]	3; 4; 4; 4	1:80

a 0.1 ml of ATS or NRS was given s.c. on each of days 9, 10, 11, 12, 13, 14 and 15 of gestation.

b Both groups were infected with 7000 pfu of SFV at gestation day 11.

c The ATS group had 5 mice.

d The NRS group had 4 mice.

Table 4.3 The effect of anti-thymocyte serum[a] on cells in the spleen and para-aortic lymph nodes of SFV-infected[b] pregnant mice, as measured by proliferative responses to antigen and mitogen in the lymphocyte stimulation test

Group	Organ	Proliferative response (cpm)		
		medium only	Concanavalin A	SFV antigen
ATS	spleen	368 ± 203[cd]	1255 ± 728[d]	207 ± 101[e]
ATS	PALN	248 ± 82[d]	842 ± 525[d]	240 ± 96[d]
NRS	spleen	1829 ± 271	43739 ± 6140	2068 ± 820
NRS	PALN	1627 ± 363	57829 ± 1637	11845 ± 2137

a 0.1 ml of ATS or NRS was given s.c. on each of days 9, 10, 11. 12, 13, 14 and 15 of gestation.

b Both groups of mice were given 7000 pfu of SFV i.p. at gestation day 11 and killed 7 days later.

c Mean ± S.E. of responses of individual organs from 5 mice in ATS group and 4 mice in the NRS group.

d The difference between the ATS value and the NRS value is significant, $p < 0.01$ (Student's t test).

e The difference between the ATS value and the NRS value is not significant.

Table 4.4 The effect of anti-thymocyte serum[a] on virus titres in the foetus, placenta and maternal serum following infection[b] with SFV during pregnancy

Treatment	Mouse	Virus titre[c]			
		Living foetus	Dead foetus	Placenta	Maternal serum
ATS	1	9.0(6)[d]	-(0)	6.5	4.3
	2	9.0(5)	9.7(1)	6.4	5.2
	3	6.8(7)	-(0)	5.9	7.0
	4	9.6(3)	9.5(4)	7.7	6.8
NRS	1	< 2.0(6)	-(0)	4.3	6.2
	2	< 2.0(6)	-(0)	5.0	6.2
	3	2.6(5)	-(0)	5.9	4.2
	4	< 2.0(7)	-(0)	5.0	6.5

a 0.1 ml of ATS or NRS was given s.c. on each of days 9, 10, 11, 12 and 13 of gestation.

b Both groups of mice were infected with 7000 pfu of SFV at gestation day 11 and killed 3 days later.

c Expressed in $\log_{10}$ pfu/g or $\log_{10}$ pfu/ml.

d The numbers in brackets are the number of foetuses present.

These results indicate that ATS treatment has a strong *in vivo* influence on T-cell mediated immune responses. Proliferation responses against mitogen and antigen in the LST were significantly reduced by ATS (Table 4.3), although there was no effect on the serum antibody titre (Table 4.2). Even so, this depression of T-cell immunity did not appear to influence the outcome of the infection because abortion occurred at a similar time to the control group (Figure 4.2). Thus, there was no evidence that the abortion which may be observed following maternal infection with SFV was caused by T-cell mediated immuno-pathological processes.

It was of interest that the titres of SFV in the foetal and placental tissues at 3 days p.i. (Table 4.4) indicate that intra-uterine infection may have been more advanced following ATS treatment, although this was not reflected in a noticeably earlier time of abortion.

DISCUSSION

Close similarities exist between the pathogenesis of *in utero* infections with SFV and RRV despite their differing clinical syndromes. With both viruses, the placenta probably became infected following a haematogenous virus spread from the site of inoculation in the mother. Foetal infection, however, occurred at least 1-2 days after the initial detection of virus in the placenta, at a time when the maternal viraemia was declining. Foetal infection was therefore less likely to have occurred from a direct seeding of virus from the maternal circulation and was probably dependent upon viral replication in the placenta. Foetal death appeared to be a direct consequence of viral replication in foetal tissue.

With both RRV and SFV, the placenta appeared to play a critical role in the pathogenesis of the *in*

utero infections. Foetal infection was probably aided by the apparent predilection of both viruses for placental tissue, and their subsequent growth to high titre in that organ. Furthermore, both viruses were able to persist at high titre within the placental tissue even though the mothers were able to mount humoral and cell-mediated anti-viral immune responses which were able to clear virus from their own tissues (see Chapter 3). This may be because placental virus is not accessible to an effective maternal immune response. For example, maternal IgM, produced in the initial phase of infection does not cross into the foetal circulation (Fahey and Barth 1965). Maternal IgG does cross the mouse placenta (Fahey and Barth 1965; Grey *et al*. 1971) but, at least in the first week after infection, does not appear to be present in sufficient amounts to clear virus from foetal tissue. The low or undetectable virus titres in some placentas and dead foetuses from mothers infected at gestation day 5 and harvested at gestation day 18 (Figures 4,8, 4.9, 4.10 and 4.11) suggest that a minimum of 13 days may be required after infection before any anti-viral effect of maternal IgG is expressed in foetal tissue.

It is also possible that virus infecting trophoblast tissue may evade recognition or elimination by the cellular component of the maternal immune response. In Chapter 1.2 it was noted that maternal transplantation immunity is not functionally expressed in the placenta, possibly because hormones produced by the foeto-placental unit are immuno-suppressive at the high concentrations that occur in the micro-environment of that organ. If this is the case, the expression of anti-viral cellular immunity may also be compromised in the placenta, and trophoblast may therefore be an immunologically "privileged" site for virus growth. This may provide an explanation for the apparent predilection of many infectious agents for placental tissue (Fox 1977). The growth and persistence of both RRV and SFV in the trophoblast in the present study

certainly supports this suggestion.

The role of the placenta as a possible barrier to the transfer of virus from the maternal circulation to the foetus is unclear. At least 1 to 2 days appears to be necessary after the initial establishment of infection within the placenta before foetal infection with RRV or SFV occurs. This is presumably the time required for viral growth through to the foetal circulation. It seems likely therefore that there is a certain threshold of virus concentration required within the placenta before foetal infection begins. These results support Fox (1977) who noted that it is probable that the placenta is able to delay the passage of infection into the foetus by acting non-specifically as a physical barrier. However, in a number of foeto-placental units in the present study, even though the placenta had an extremely high concentration of virus 7 or more days after maternal inoculation, no evidence of infection could be found in the foetus. A similar situation of placental infection without foetal infection has been described in other diseases including human rubella (Alford et al. 1964) and CMV infections (Hayes and Gibas 1971), as well as in animal infections with LCMV (Mims 1968), CMV (Johnson 1969) and IBRV (Kendrick et al. 1971; Kendrick 1973). Thus, mechanisms appear to exist in some foeto-placental units which either prevent virus from reaching the foetus from the placental foci, or render the foetus immune to infection. One non-specific mechanism with the potential to restrict but not necessarily inhibit virus growth through the placenta is interferon which, during a normal mouse pregnancy, progressively concentrates in placental tissue (Fowler et al. 1980). This may explain the localised nature of the virus foci in the trophoblast with both RRV and SFV, although this could also be linked to the location and number of the susceptible target cells. Furthermore, Murasko and Blank (1980) have demonstrated that exogenous interferon can exert a trans-placental anti-viral effect in mice with a high

rate of spontaneous *in vivo* leukaemia virus production. Although it is therefore possible that interferon may be involved with either preventing virus growing through the placenta or inhibiting the replication of virus after reaching the foetus, it is difficult to understand why this or any other mechanism could be functional in some but not other foeto-placental units.

It seems unlikely that these apparently uninfected foetuses with high concentrations of virus within their placental tissue have recovered from infection because:

a) as noted above, maternal IgG, which does cross to the foetus, may only exert an anti-viral effect in the foetus and placenta after 13 days p.i.;

b) the mouse foetus appears to be immunologically incompetent (Silverstein 1972), and the full development of the mouse immune response may only be completed after birth (Makinodan and Petersen 1962; Dalmasso *et al*. 1963; Chiscon and Golub 1972). Furthermore, both RRV and SFV are uniformly lethal for neonatal mice, even in doses of 100 pfu or less.

c) there was no evidence of *in utero* growth retardation in the form of lower body weight in the foetuses that were spared from an infection with RRV.

In contrast to RRV infection, SFV invariably caused abortion in mice infected at gestation days 10 or 11. Results presented in this and the preceding Chapter indicate that the time of abortion induced by infection with SFV and the time of onset of the T-cell mediated anti-viral immune response coincide. Because the T-cell mediated immune response can cause tissue

destruction that may even prove fatal if there is a massive synchronous destruction of functionally important cells (Berger and Blanden 1981; Doherty and Bennink 1981), mothers infected with SFV were immuno-suppressed to investigate whether the abortion could be attributed to immuno-pathological mechanisms. Possibly the best way to induce maternal immuno-suppression without using irradiation or anti-mitotic agents which may have a deleterious effect on the pregnancy is to use ATS. ATS has been shown to depress the primary cellular response to a number of viral agents, and appears to act against recirculating thymus-derived small lymphocytes which are either killed or opsonised, then cleared by the reticuloendothelial system in the liver (Lance et al. 1973). Thus, ATS treatment results in a lymphopenia along with a selective depletion of small lymphocytes from the para-cortical areas of lymph nodes and the peri-arteriolar regions of splenic follicles (Taub 1969). Mims (1969) used ATS to investigate whether the maternal death and abortion induced by injection of LCMV into pregnant mice was immuno-pathological in nature. It was found that potent ATS administered repeatedly did, in fact, reduce the incidence of death and abortion, and the pregnancies proceeded to term. However, because the cellular infiltrates which may have been expected to accompany a significant local cellular immune response were not evident in the placenta, it was suggested that the abortion caused by LCMV infection was possibly secondary to a systemic physiological disturbance in the mother which was not compatible with the progression of the pregnancy. In the present study, T-cell proliferation responses to both viral antigen and Con A were markedly depressed in the spleen and PALN following ATS treatment (Table 4.3). However, as previously noted by Lance et al. (1973) in similar circumstances, the anti-viral antibody titre was unaffected. This depression of T-cell anti-viral responses appeared to have no effect on the occurrence or time of abortion, suggesting that the premature interruption of pregnancy following

infection with SFV was not T-cell mediated. It must be noted, however, that Mims (1969) demonstrated that the ability of ATS to prevent maternal death or abortion after LCMV infection in mice was proportional to the potency of the preparation, and it is therefore possible that a more potent ATS in the present study may have produced a different result. Against this, Taub (1969) noted that the potency of ATS was proportional to the degree of lymphocyte depletion from lymphoid tissue. The significant depression of both the spleen and lymph node responses after treatment in the present study suggests that the ATS preparation used was highly potent.

Although T-cell depletion did not appear to have any noticeable effect on the time that abortion occurred after maternal infection with SFV, the results presented in Table 4.4 indicate that the in utero infection may have been more advanced in the ATS group. This suggests that the onset of the maternal viraemia, and therefore placental infection, was earlier and of greater magnitude in the absence of sensitised T-cells. The reason why the accelerated in utero infection associated with ATS treatment was not reflected as an early abortion is not clear. Perhaps with larger groups of mice and more frequent inspections of them following infection, a difference in the time of abortion following ATS treatment may have been apparent.

Other clues as to the reason why abortion occurs following infection of 11-day pregnant mice with SFV comes from an examination of Figures 4.10 and 4.11. It appears that the outcome of SFV infection during pregnancy depends upon the timing of infection with regard to the stage of gestation. Infection with SFV at day 10 or day 11 of gestation always results in abortion, and this appears to be preceded by uniform foetal infection. However, infection with SFV earlier in gestation does not always result in abortion, and in those pregnancies which were not interrupted at

least one uninfected foetus was detected. Thus, the outcome of SFV infection in this latter group of mothers resembles that observed following infection of 11-day pregnant mice with RRV (Figure 4.3). These results indicate that the abortion caused by SFV is only precipitated by uniform foetal infection, and are in accord with Arthur (1975) who noted that in polytocous animals, provided at least one viable foetus is present within a pregnancy, normal gestation and parturition will occur. It is presumed that the abortion caused by SFV is somehow linked to a perturbation in foeto-placental hormone production. The fact that abortion only occurs in some infected mothers suggests that it is not caused by adverse effects of the infection on maternal physiological function. Furthermore, although it is difficult to prove whether abortion could result from a virus-induced impairment of placental function, the high titres of SFV in all placentas of the 2 mice that did not abort following infection at day 7 (Figure 4.10) tend to indicate that this is not the case. Whatever the mechanism involved, the time interval between foetal infection and abortion is short and, according to Figure 4.7, may be only 12 hours.

As noted above, the pattern of foetal infection in the mice that do not abort following SFV inoculation is similar to that observed with RRV, in that each pregnancy contains some dead and some uninfected, live foetuses. This suggests that the mechanisms involved in determining which foetuses are infected and which are spared may be similar for both viruses. However, the results presented in Figures 4.9 and 4.11 suggest that SFV may be more efficient than RRV at causing foetal infection at most stages of gestation. In fact, at gestation days 10 or 11 all foetuses seem to be fatally infected, with abortion occurring as a consequence. Thus, the reason why abortion was not observed with RRV may have been that the virus rarely caused uniform foetal infection. In the few cases where all foetuses

were found to be infected with RRV, there was a possibility that the animal may have aborted after the time of harvest. Nevertheless, Aaskov et al. (1981a) reported 1 abortion out of 150 pregnant mice infected with RRV, and it is of interest that virus was detected in all the aborted foetuses.

The results presented by Aaskov et al. (1981a) provide an interesting comparison with some of the results of the present study. They also infected pregnant CBA/H mice with the T48 strain of RRV, however they used older mice (10-16 weeks of age), a larger dose of virus (10^6 pfu), and a different route of inoculation (i.v.). The major differences between the 2 studies are:

a) although Aaskov et al. (1981a) noted that infection occurred in a proportion of foetuses in each pregnancy, some of these foetuses were thought to have recovered in utero;

b) Aaskov et al. (1981a) detected foetal infection much earlier than in the present study, at 1 day p.i.;

c) they did not observe consistent placental infection at any time after maternal infection at gestation day 10. In fact, on each of days 1 to 9 p.i. only 50% (approximately) of the placentas contained virus.

Some of the differences between these two studies may be attributable to the different dosage and route of infection. A large i.v. dose of virus would create an immediate maternal viraemia facilitating early seeding of the virus to the placenta, and possibly the foetus. For this reason, the dosages used throughout the present study were chosen to be high enough to uniformly infect all animals but it was hoped that a viraemia would only result after virus replication.

The other disparities between the results of Aaskov et al. (1981a) and those of the present study may be due to a divergence of virulence and tropisms between virus stocks. Whereas the T48 strain used in the present study was in its 13th mouse brain passage, that used by Aaskov et al. (1981a) was in its 6th mouse carcass passage.

In summation, infection of pregnant mice with either RRV or SFV can result in viral growth in both placental and foetal tissue which ultimately leads to foetal death. This large intrauterine viral antigen load may be responsible for the perturbation of the mothers' anti-viral immune response observed in the results of Chapter 3. The mechanisms involved in the pathogenesis of the in utero infections are, however, not clear. In particular, the reason why some foetuses are spared from infection whereas others within the same pregnancy succumb is puzzling, especially when it is considered that because the pregnancy is inbred the foetuses should all be genetically identical. This is investigated further in the next Chapter.

CHAPTER 5

THE NATURE OF THE MECHANISMS THAT DETERMINE WHICH FOETUSES ARE INFECTED AND WHICH ARE SPARED FOLLOWING MATERNAL INFECTION WITH EITHER ROSS RIVER VIRUS OR SEMLIKI FOREST VIRUS

INTRODUCTION

In the previous Chapter, virological aspects of the pathogenesis of *in utero* infections with RRV and SFV were described. The placenta, in particular, played a critical role in determining the outcome of infection. Foetal infection was aided by the apparent predilection of both viruses for placental trophoblast, and their subsequent growth and persistence at high titre in that organ. However, following maternal infection with either RRV or SFV at certain stages of gestation, a proportion of the foetuses in each pregnancy were always spared, even though others were infected and rapidly died. The sparing of one or more foetuses resulted in the continuation of the pregnancy rather than abortion.

The experiments described in this Chapter were designed to determine whether some foetuses are spared because virus does not migrate from their placentas, or whether they are specifically protected from virus infection by an immunological process.

RESULTS

Are foetuses spared from *in utero* infection with RRV because they are protected against the infection?

Foetuses spared from *in utero* infection with RRV were experimentally challenged with RRV after birth in order to assess their immune status. To avoid the influence of antibody-enriched colostrum and milk this challenge was performed on foetuses delivered by caesarian section and fostered onto uninfected mothers.

Preliminary experiments (not presented) indicated that fostering was more successful if the caesarian sections were performed at gestation day 19, rather than day 18. Therefore, 3 11-day pregnant mice were infected i.p. with 2600 pfu of RRV, and 8 days

later their 6 living foetuses were removed by caesarian section and fostered onto 2 uninfected CBA/H mothers which had just given birth naturally. These, and a fostered control litter from a 19-day pregnant, uninfected mother, were challenged s.c. 24 hours later with 100 pfu of RRV. The results (Table 5.1) show that all control neonates died with a mean survival time of 2.8 days, whereas all neonates delivered from infected mothers survived challenge.

The duration of this protection was evaluated. Eleven-day pregnant mice were infected i.p. with 2600 pfu of RRV, and 8 days later their living foetuses were delivered by caesarian section and fostered onto uninfected mothers. Groups of these neonates and controls from uninfected mothers were infected i.p. with 1300 pfu of RRV at either 1, 3, 6 or 9 days after fostering. Control neonates were susceptible to infection at all times, although the mean survival time increased with age (Table 5.2). All neonates from infected mothers were resistant to infection at days 1, 3 and 6 of life but not day 9, although the mean survival time of the latter group was 2.2 days longer than the control group. These results indicate that the foetuses that are spared from in utero infection with RRV are specifically protected against the virus, but this protection is only short-lived.

The nature of the protective mechanisms

The transient nature of the foetal protection against RRV challenge suggested that it was mediated by antibody passively acquired from the mother. Serum from spared foetuses was therefore separated into its different immunoglobulin fractions to establish whether anti-RRV activity was present and, if so, what antibody classes were involved.

A group of 8 11-day pregnant mice were infected i.p. with 2600 pfu of RRV, and 7 days later they were killed and their sera pooled. There were 28 dead and 41 live foetuses present. Serum was obtained from the live foetuses (hereafter termed infected foetal serum) by taking whole

Table 5.1 The responses of foetuses[a] which survive maternal infection with RRV to post-natal challenge with homologous virus

Mothers	Number of Challenged Neonates [b]	Number Surviving Challenge	Mean survival time (days)
Infected[c]	6	6	-
Uninfected[d]	5	0	2.8

a Foetuses were delivered by caesarian section from either RRV-infected or uninfected 19-day pregnant mothers and fostered onto uninfected mothers.

b Fostered neonates were challenged s.c. with 100 pfu of RRV.

c Mothers were infected i.p. with 2600 pfu of RRV at gestation day 11.

d Uninfected control mothers.

Table 5.2 The duration of protection against post-natal challenge with RRV in the foetuses[a] which survive maternal infection[b]

Time of challenge[c] (Day of life)	Neonates from infected mother			Neonates from uninfected mother		
	Number challenged	Number survived	M.S.T.[d] (days)	Number challenged	Number survived	M.S.T. (days)
1	6	6	-	26	0	3.2
3	3	3	-	3	0	3.3
6	4	4	-	4	0	3.4
9	4	0	8.0	4	0	5.8

a Foetuses were delivered by caesarian section from 19-day pregnant mice and fostered onto uninfected mothers.

b Mothers were infected i.p. with 2600 pfu of RRV at gestation day 11.

c All neonates which survived delivery were challenged i.p. with 1300 pfu of RRV at different times after birth.

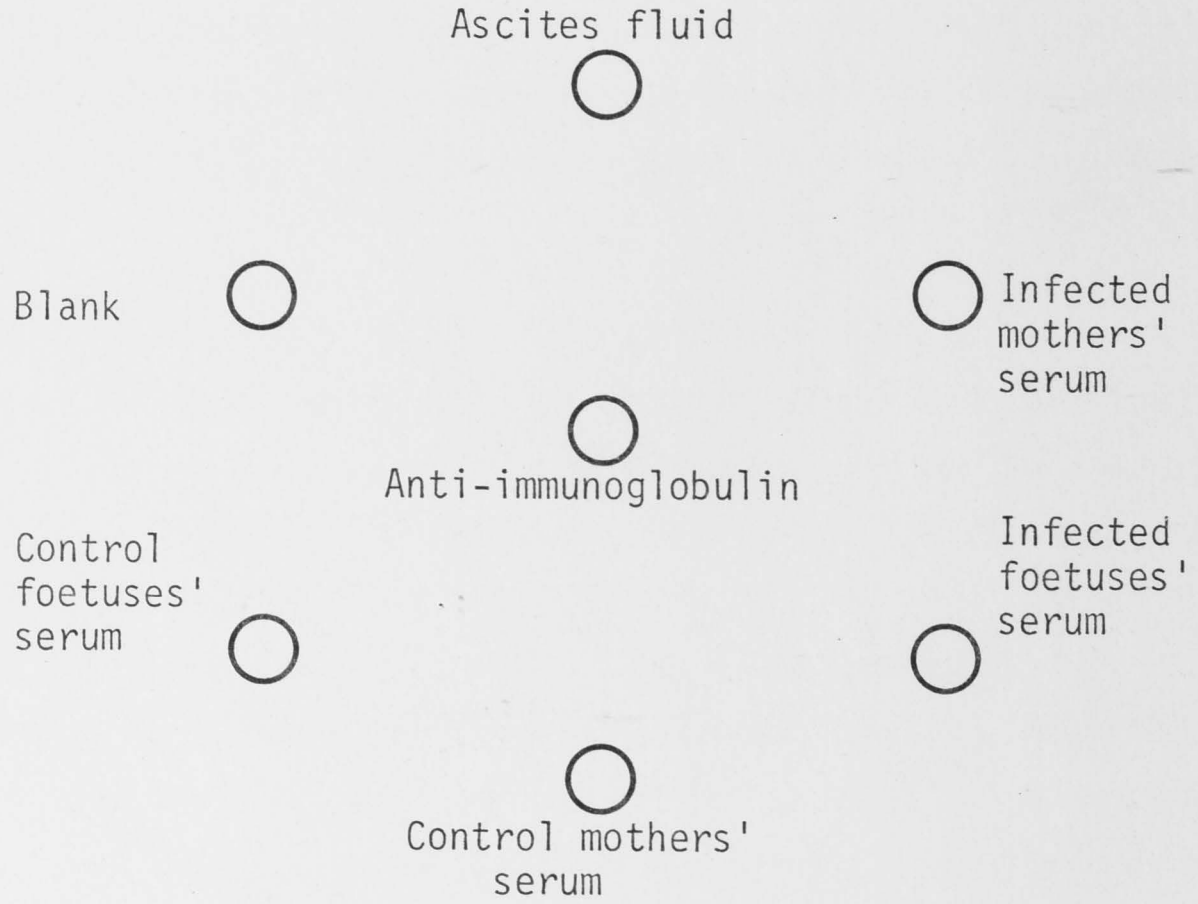
d M.S.T. = Mean Survival Time.

blood (approximately 50 ul per foetus) into 2 ml of saline and gently centrifuging to remove erythrocytes. A further 9 uninfected mothers and their 78 living foetuses were also bled at the same stage of gestation. The immunoglobulins present in these 4 pools, and in hyper-immune ascites fluid made against RRV as a known high-titred antiserum, were identified by the gel diffusion test (Figure 5.1, Table 5.3). In the foetal sera, IgM and IgA were not detectable, and only faint precipitin lines against IgG2b were observed. All 6 immunoglobulin isotypes were detected in the ascites fluid and the 2 maternal serum pools. It must be noted that more than 1 precipitin line was detected against anti-IgM and anti-IgA in Figure 5.1 (and Figure 6.1). This is presumably because these antisera contain contaminating specificities against other serum proteins. For example, antisera raised against IgM may contain contaminating specificities against α-macroglobulin (Ey 1973).

All 4 serum pools, plus the ascites fluid were fractionated on a Sepharose CL-4B-protein A column. The amount of serum protein loaded onto the column was: ascites fluid, 121.5 mg; infected mother, 121.6 mg; infected foetus, 10.6 mg; control mother, 122.8 mg; control foetus, 28.2 mg. The optical density of each fraction in the Coomassie Blue reaction is presented in Figure 5.2, and is proportional to the protein content. Because the elution pattern obtained with the hyper-immune ascites fluid indicated that 4 major protein peaks were present, for each of the other serum pools the fractions in the vicinity of these peaks were pooled, concentrated, and the immunoglobulin subclasses identified (Table 5.3). In the ascites fluid all detectable IgA, and a proportion of the IgM, passed through the column without binding and eluted with the other serum constituents in peak 1. Some IgM appeared to bind to the column, being eluted with IgG1 in peak 2, and IgG2a/IgG3 in peak 3. IgG2b was present in peak 4. The binding of a proportion of the IgM to the column was surprising, but has been reported previously by Field et al. (1980).

Figure 5.1 Gel diffusion test: The identity of the immunoglobulin isotypes present in non-fractionated serum pools.

All tests were set up as follows:



Anti-immunoglobulins were:

Test A = Anti-IgM

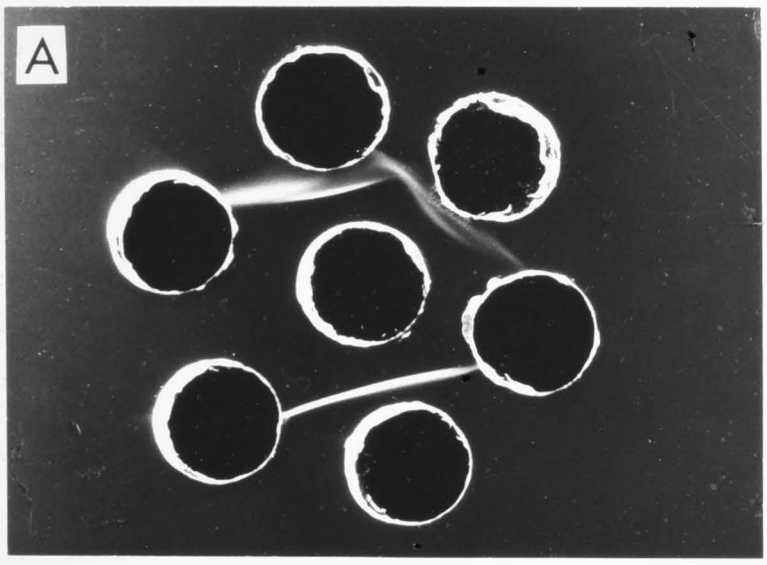
Test B = Anti-IgA

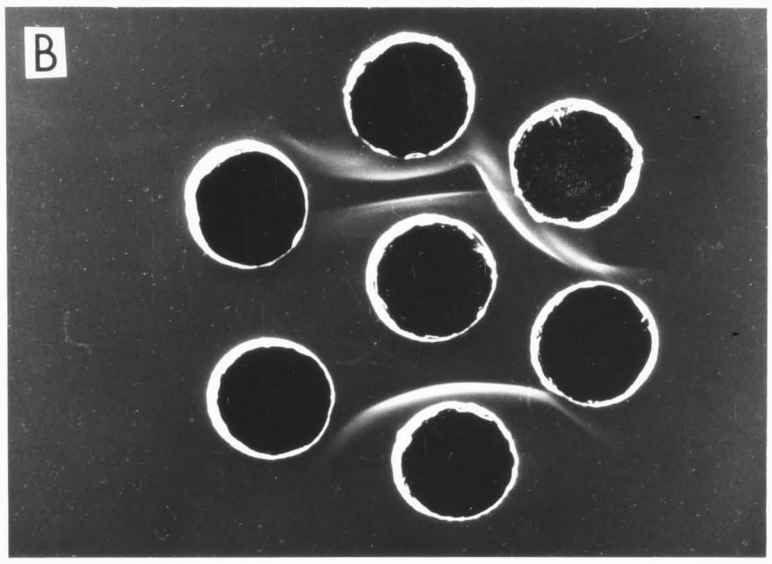
Test C = Anti-IgG1

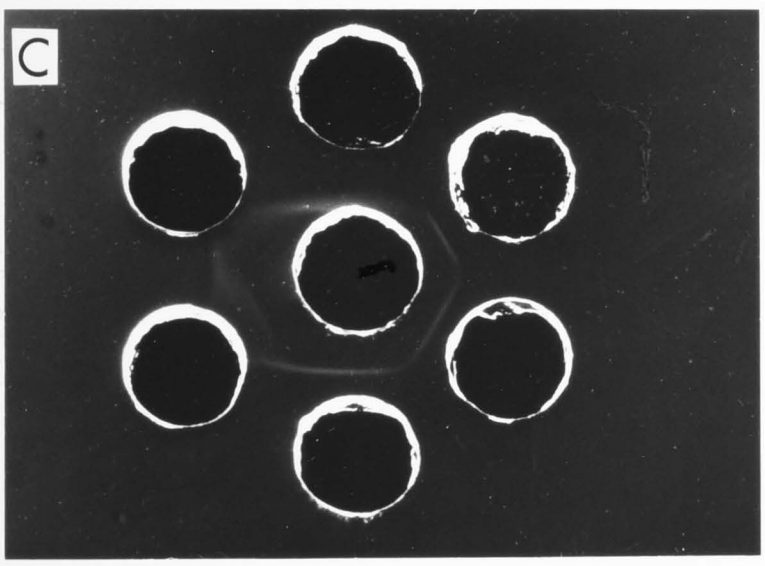
Test D = Anti-IgG2a

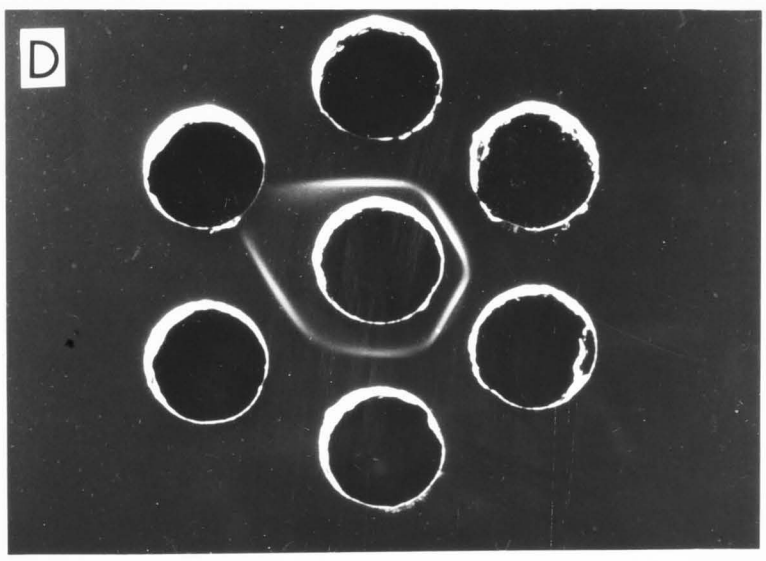
Test E = Anti-IgG2b

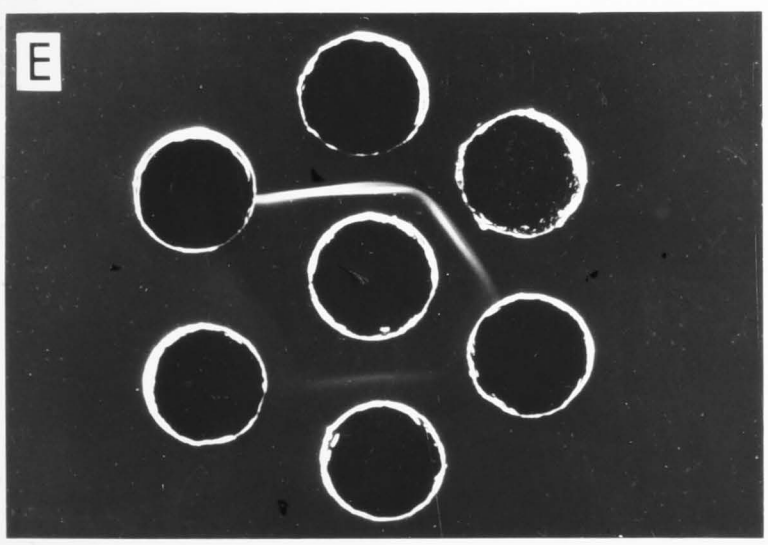
Test F = Anti-IgG3


A


B


C


D


E

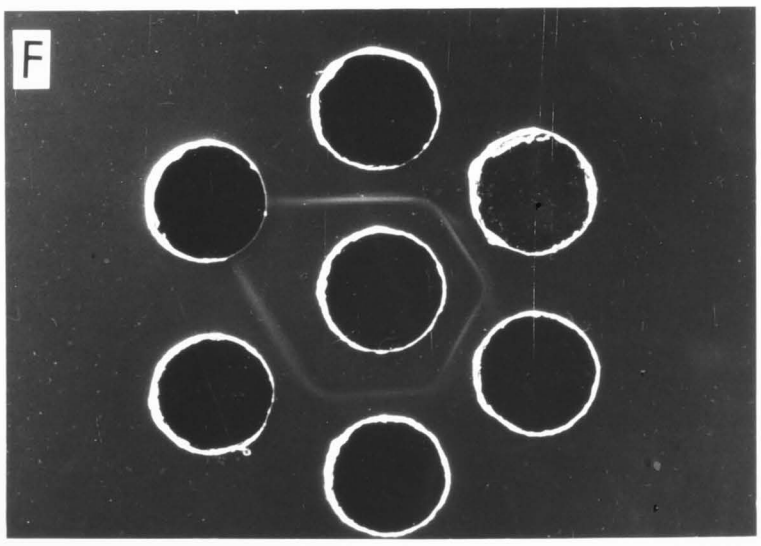

F

Table 5.3 The identity of immunoglobulins detected in various fractions[a] of maternal and foetal sera and hyper-immune ascites fluid

Peak Number	Fractions[b] (Approx.)	Antibody detected[c]				
		Ascites fluid	Infected[d]		Control[d]	
			mother	foetus	mother	foetus
1	1 - 17	A, M	A, M	-	A, M	-
2	20 - 40	M, G1	G1	-	G1	G1
3	44 - 54	M, G2a, G3	G2a, G3	G2a	G2a	G2a
4	57 on	G2b	G2b	-	-	-
Non-fractionated serum		A, M, G1, G2a, G2b, G3	A, M, G1 G2a, G2b, G3	G1, G2a, G2b, G3	A, M, G1, G2a, G2b, G3	G1, G2a, G2b, G3

a Sera were fractionated on a Sepharose CL-4B-protein A column using a linear pH gradient. The elution patterns are displayed in Figure 5.2.

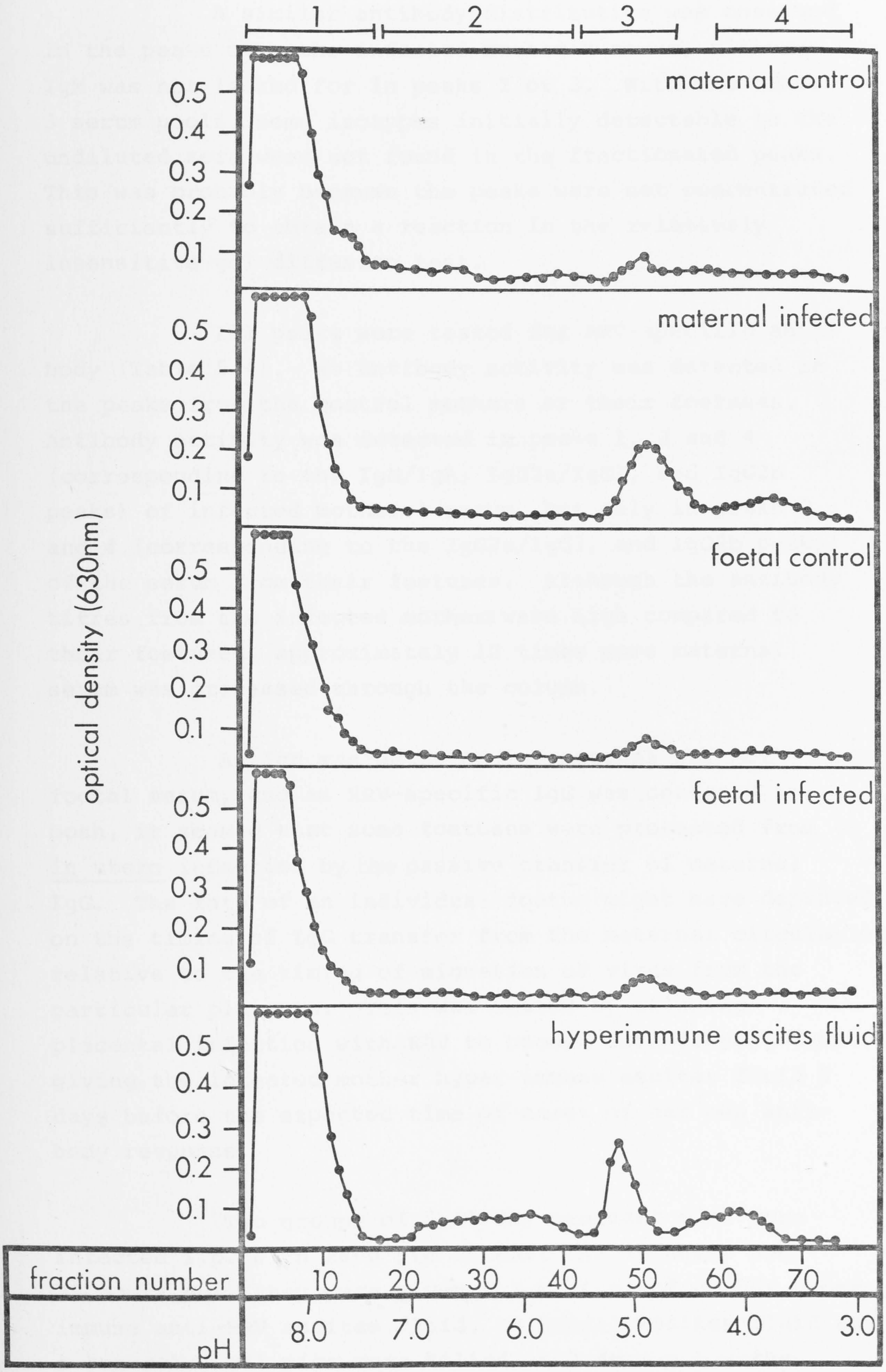
b Peak sizes varied slightly between sera but always encompassed the relevant fractions.

c In the gel diffusion test. Immunoglobulin class or subclass presented.

d 11-day pregnant mice were infected i.p. with 2600 pfu of RRV. At 7 days p.i. they and their living foetuses were bled. Control sera were taken from uninfected mothers and their foetuses at the same stage of gestation.

Figure 5.2 Elution patterns obtained with various serum samples after fractionation on a Sepharose CL-4B-protein A column.

11-day pregnant mice were infected i.p. with 2600 pfu of RRV. Serum was taken from the mothers and all living foetuses 7 days later. Control sera were from uninfected mothers and their foetuses at the same stage of gestation. The optical density of each fraction after treatment with Coomassie Blue is plotted, and is proportional to the protein content. The approximate positions of the 4 major peaks detected in the ascites fluid are shown at the top of the figure.


1
2
3
4
maternal control
maternal infected
foetal control
foetal infected
hyperimmune ascites fluid
0.5
0.4
0.3
0.2
0.1
optical density (630nm)
fraction number
10
20
30
40
50
60
70
pH
8.0
7.0
6.0
5.0
4.0
3.0

A similar antibody distribution was observed in the peaks from the infected mother's serum, although IgM was not looked for in peaks 2 or 3. With the other 3 serum pools, some isotypes initially detectable in the undiluted sera were not found in the fractionated peaks. This was probably because the peaks were not concentrated sufficiently to obtain a reaction in the relatively insensitive gel diffusion test.

All peaks were tested for RRV-specific antibody (Table 5.4). No antibody activity was detected in the peaks from the control mothers or their foetuses. Antibody activity was detected in peaks 1, 3 and 4 (corresponding to the IgM/IgA, IgG2a/IgG3, and IgG2b peaks) of infected mothers' serum, but only in peaks 3 and 4 (corresponding to the IgG2a/IgG3, and IgG2b peaks) of the serum from their foetuses. Although the antibody titres from the infected mothers were high compared to their foetuses, approximately 12 times more maternal serum was processed through the column.

As IgM was detected in maternal but not foetal serum, and as RRV-specific IgG was detected in both, it seemed that some foetuses were protected from in utero infection by the passive transfer of maternal IgG. The fate of an individual foetus might have depended on the timing of IgG transfer from the maternal circulation relative to the timing of migration of virus from the particular placenta. This was tested by allowing placental infection with RRV to become established, then giving the infected mother hyper-immune ascites fluid 2 days before the expected time of onset of her own antibody response.

Two groups of 5 11-day pregnant mice were infected i.p. with 2600 pfu of RRV. At 48 hours and 72 hours p.i. they were given 0.2 ml i.p. of either hyper-immune anti-RRV ascites fluid, or normal ascites fluid as a control. All mice were killed at 7 days p.i., the numbers of dead and live foetuses noted, and placentas

Table 5.4 HI antibody in peaks from infected[a] and control mothers and their foetuses

Peak[b] Number	HI titre[c]			
	Infected		Control	
	Mother	Foetus	Mother	Foetus
1	128	<10	< 10	< 10
2	< 2	< 2	< 2	< 2
3	1024	32	< 2	< 2
4	64	4	< 2	< 2
Undilute serum	256	ND[d]	< 2	< 2

a 11-day pregnant mice were infected i.p. with 2600 pfu of RRV. At 7 days p.i. serum was obtained from them and their living foetuses. Control sera were taken from uninfected mothers and their foetuses at the same stage of gestation.

b These peaks correspond to those in Figure 5.2 and Table 5.3.

c Reciprocal of the titre per ml.

d ND = Not Done.

from each mother were pooled and assayed for virus (Table 5.5). In the mice given normal ascites fluid there were 20 live and 18 dead foetuses, compared with 45 live and only 1 dead foetus in the group given immune ascites fluid. No virus was detected in the latter foetus so this death was probably non-specific. All placental pools contained virus at high titre. Thus, all foetal infection could be prevented using immune ascites fluid even though virus was present in high titre in placental tissue.

The effect of infection with RRV during an outbred pregnancy

Experiments described so far in this study have only investigated the effects of maternal virus infection in inbred pregnancies. As this type of mating does not occur naturally, the effect of infection with RRV during an outbred or allogeneic pregnancy was examined.

Five CBA/H females pregnant to Balb/c males were infected i.p. with 2600 pfu of RRV at gestation day 11, and killed 7 days later. Live and dead foetuses, as well as their placentas, were pooled from each mouse for virus assay. Two living foetuses were fostered onto an uninfected mother and challenged s.c. with 100 pfu of RRV 24 hours after caesarian section.

There was no difference between the outcome of maternal infection in an outbred pregnancy compared to what has been described previously in the inbred CBA/H x CBA/H pregnancy. All dead foetal pools and placental pools contained virus at high titre (Table 5.6). The three pools of live foetuses had no detectable virus. Of the 2 fostered foetuses, one died overnight from unknown causes, whereas the other was protected against post-natal challenge with RRV.

Table 5.5 The effect of normal and immune ascites fluids on *in utero* infection with RRV[a]

Group[b]	Mouse number	Number of foetuses		Placental virus titre (log_{10} pfu/g)
		Live	Dead	
Normal ascites fluid	1	6	4	6.75
	2	6	1	6.58
	3	2	5	7.76
	4	3	4	6.36
	5	3	4	6.60
Immune ascites fluid	1	10	0	6.18
	2	9	1[c]	6.49
	3	10	0	6.79
	4	9	0	6.54
	5	7	0	5.39

a 11-day pregnant mice were infected i.p. with 2600 pfu of RRV.

b 0.2 ml of either normal or immune ascites fluid given i.p. at 48 hours and 72 hours p.i.

c No virus was detected in this foetus.

Table 5.6 The effect of infection with RRV during an out-bred[a] pregnancy

Mouse	Living foetus		Dead foetus		Placental Titre[b]
	Number	Titre[b]	Number	Titre[b]	
1	1	< 1.7	6	7.60	6.70
2	2	< 1.7	6	6.90	6.88
3	2[c]	< 1.7	3	5.48	6.78
4	1[c]	ND[d]	5	7.79	6.58
5	0	-	6	6.30	6.86

a CBA/H females pregnant to Balb/c males were infected i.p. with 2600 pfu of RRV at gestation day 11, and killed 7 days later.

b Virus titre ($\log_{10}$ pfu/g).

c One foetus from each mouse was fostered onto an uninfected mother. Of the 2 foetuses, one died within 12 hours and the other survived > 8 days after s.c. challenge with 100 pfu of RRV.

d ND = Not Done.

The post-natal death rate following maternal infection with RRV at gestation day 11

Aaskov et al. (1981a) described a high post-natal death rate in the young of mothers infected with RRV. This would not be predicted from the results of the present study because all foetuses that survive to parturition should be protected against virus infection. The incidence of post-natal deaths in neonates born naturally to mothers infected with RRV at gestation day 11 was therefore examined. Because this entailed frequent handling of the babies, and consequent disturbance of the mother, a group of control mothers injected with saline was included.

Two groups of 8 11-day pregnant mice were injected i.p. with either 0.1 ml of normal saline or 2600 pfu of RRV. All mothers were individually caged and examined regularly for the number of live births, still births and mummified foetuses. The times of any post-natal deaths were noted (Table 5.7). It must be emphasised that some of the totals, especially for the mummified foetuses and still births, may be lower than the actual number because of cannibalism by the mother. All available still births and post-natal deaths, but not mummified foetuses, were assayed for virus.

There were 15 live births and 4 still births in the infected group, and 52 live births and 6 still births in the saline group. Virus was detected in 3 of of the 4 still births from the infected mothers. There appeared to be a higher rate of post-natal deaths in the infected group (6 deaths out of 15 live births = 40%), compared to the saline group (9 deaths out of 52 live births = 17%).

The role of maternal antibody in the outcome of in utero infection with SFV

Concentration was then focused on the role,

Table 5.7 The incidence of post-natal death following maternal inoculation[a] with either saline or RRV at gestation day 11

Group[a]	Number observed at birth			Number of post-natal deaths in the period (hr after birth)					
	live births	still births	mummified foetuses	3-8	8-24	24-48	48-72	72-96	96-120
Saline	52	6[d]	0	1[d]	6[d]	2[d]	0	0	0
RRV-infected	15	4[b]	14[d]	3[c]	2[c]	0	0	0	1[c]

a Groups of 8 11-day pregnant mice were injected i.p. with either 0.1 ml of normal saline or 2600 pfu of RRV.

b Virus detected in 3 of the 4 still births.

c No virus detected.

d Not assayed for virus.

if any, of maternal antibody in the pathogenesis of *in utero* infection with SFV. Results presented earlier in this Chapter suggested that hyper-immune ascites fluid administered to pregnant mice after placental infection with RRV had established, but before the onset of the mother's antibody response, protected all foetuses from infection. A similar experiment was therefore performed with SFV.

Two groups of 5 10-day pregnant mice were infected i.p. with 7000 pfu of SFV. At 48 hours and 72 hours p.i. they were given 0.2 ml i.p. of either normal ascites fluid or immune anti-SFV ascites fluid. Of the mice given normal ascites fluid, all aborted between days 4 and 6 p.i. (Table 5.8). Only one abortion (at day 7 p.i.) was observed in the mice given immune ascites fluid. The mice that had not aborted were harvested at gestation day 19 (i.e., day 9 p.i.). No dead foetuses were observed. Of 7 live foetuses which were successfully fostered onto uninfected mothers, all survived s.c. challenge with 7000 pfu of SFV. Of the 4 placental pools, 3 had detectable titres of virus. Presumably, the haematogenous spread of virus to the placenta was prevented in the mother that had no detectable placental virus, and did not abort (Immune ascites group, number 5).

Thus, similar to the results obtained earlier with RRV, hyper-immune ascites fluid is capable of protecting foetuses against *in utero* infection with SFV, even though high titres of virus may be present in the placental tissue. When foetal protection occurs, abortion is not observed.

DISCUSSION

One aim of the investigations reported in this Chapter was to elucidate why some foetuses died *in utero* whereas others were spared following maternal infection with RRV. Initial evidence indicated that

Table 5.8 The effects of normal and immune ascites fluids on *in utero* infection with SFV[a]

Group[b]	Mouse number	Number of foetuses		Placental virus titre (log_{10} pfu/g)[c]
		Live	Dead	
Normal ascites fluid	1	Aborted 4 days p.i.		ND[d]
	2	" 5 "		ND
	3	" 5 "		ND
	4	" 5 "		ND
	5	" 6 "		ND
Immune ascites fluid	1	Aborted 7 days p.i.		ND
	2	8	0	8.45
	3	8	0	6.30
	4	3	0	4.15
	5	6	0	< 1.7

a 11-day pregnant mice were infected i.p. with 7000 pfu of SFV.

b 0.2 ml of either normal or immune ascites fluid given i.p. at 48 hours and 72 hours p.i.

c Placental pools from all mice that had not aborted were assayed for virus content at 9 days p.i.

d ND = Not Done. Aborted tissues were not available for virus assay.

these spared foetuses were protected against virus challenge. Two observations suggested that the protection was mediated by passively transferred maternal antibody rather than an active foetal immune response. Firstly, the offspring were only resistant to the lethal effects of homologous challenge for a short period after birth. The waning of protection between the 6th and 9th day of life (Table 5.2) may have been because the half-life of immunoglobulin in the mouse is between only 1.0 and 5.0 days (Fahey and Robinson 1963; Grey et al. 1971). Secondly, anti-RRV antibody activity was only observed in the IgG-containing peaks and was not associated with IgM or IgA. In the mouse, IgG is the only maternal antibody class transferred pre-natally to the foetus (Fahey and Barth 1965; Grey et al. 1971).

If foetuses are spared from in utero infection with RRV because they have been passively immunised by specific maternal IgG, why do other foetuses within the pregnancy not get the benefit of this protection? The answer to this may be linked to the timing of foetal infection relative to the time of onset of specific maternal IgG production. The latter time is not known accurately but would be earlier than day 7 p.i. when strong anti-RRV activity was detectable in the maternal IgG2a/IgG3, and IgG2b peaks (Table 5.4). It seems likely that passive foetal immunisation would commence within 6 hours of the release of specific IgG into maternal serum (Carretti and Ovary 1969). Foetal infection, on the other hand, probably occurred over a longer period. Although the time between in utero infection and death appears to be approximately 2 days (from Figure 4.3), live, infected foetuses were observed on days 3, 4, 5 and 6, but not day 7 p.i. This suggests that foetal infection actively occurred from day 3 p.i. up to day 5 p.i. The reason why all foetuses did not become infected at the same time may be because the concentration of virus in individual placentas varied considerably at each harvest time (Figure 4.2), indicating that either the time of

initial trophoblast infection, or the rate of growth of virus through the placenta was not uniform for each foetus. Because the onset of passive foetal immunisation may have coincided with the time of active foetal infection, it is possible that the fate of each foetus depended on whether protective IgG or virus crossed the placenta first. Thus foetuses whose placental tissue had a high concentration of virus may have been more likely to be infected before the onset of transfer of passive protection, whereas others, with lower placental virus loads, may have been protected before virus was able to grow through the placenta. The observation that there was little or no difference in virus concentration in the placentas of dead foetuses compared to those of live foetuses at days 5 to 7 p.i. (Figure 4.2) may be irrelevant to this discussion because whether or not a foetus will be protected or infected *in utero* has probably been determined by then.

The results presented in Table 5.5 provide support for this hypothesis. The effect of passive immunisation against RRV earlier than would usually occur was to protect all foetuses from infection despite the continued presence of virus at high titre in the placenta. Thus, at least in this experimental system, passive therapeutic immuno-therapy appeared to be highly effective for foetal protection, providing it was performed soon after maternal infection.

An attempt to further substantiate this hypothesis involved an investigation of the effect of RRV infection in 11-day pregnant, congenitally athymic nude CBA/H mice. Because these mice are T-cell deficient, they form IgM but not IgG in response to a viral infection (Bradish *et al*. 1979). In theory, therefore, all foetuses should be infected with RRV *in utero* because passive immunisation could not occur. However, this experiment was not completed. A major problem was related to the low fertility of nude mice which made it impossible to achieve a successful

pregnancy using an economically viable number of mice.

The role of the protective maternal IgG in foetal tissue is unclear. Sissons and Oldstone (1980) noted that antibody plays an important role in the termination of a primary viral infection by the neutralisation of free virus particles and the lysis of infected cells before the release of progeny virus. In the present study, although maternal IgG presumably conferred protection by neutralising all virus moving from the placental foci into the foetal circulation, antibody alone did not appear to be able to mediate recovery from in utero infection. Indeed, it seemed probable that once infection had established in the foetus, death was inevitable. The inability of maternal IgG to clear infection from foetal or placental tissue indicates that intra-cellular virus is not eliminated, and that the spread of virus probably occurs between contiguous cells. It therefore seems that the conclusion drawn in Chapter 4, that maternal IgG is able to neutralise virus in placental or foetal tissue, but only after a minimum of 13 days p.i. is incorrect. The reasons for the decrease in virus titres in these tissues after infection with RRV or SFV at gestation day 5 and harvest 13 days later (Figures 4.8, 4.9, 4.10 and 4.11) are not known but may include autolysis, or a decrease with time, in the number of susceptible target cells.

Of relevance to this discussion is the postulate of Bell and Billington (1980) that, in the mouse, complement-fixing, cytotoxic IgG2a production may be suppressed during pregnancy in favour of the production of non-complement-fixing IgG1. This was considered to be beneficial for the survival of the allogeneic foetus, especially because the IgG1 was thought to be responsible for immunological enhancement

of foetal antigens. The results of the present study, however, suggest that, at least in viral infections during pregnancy, this may not be the case because anti-RRV antibody activity was not detected in the IgG1 peak of maternal serum (Table 5.4). Thus, even though the foetal mouse produces at least some of the complement components (Tachibana and Rosenberg 1966), it appears that either complement mediated antibody cytotoxicity does not occur in the infected foetus or placenta, or it is not an important mechanism for the clearance of virus from infected tissue.

Aaskov et al. (1981a) noted that infection of pregnant mice with RRV was associated with a high post-natal death rate in the offspring. Virus was not isolated from the dead infants, although in approximately 20% viral antigen could be demonstrated in cells lining the aqueduct of Sylvius and the choroid plexus. Similar results were observed in the present study, although immuno-fluorescence studies were not performed. RRV was not expected to cause either still births or post-natal deaths following maternal infection at gestation day 11 because by gestation day 18 (i.e., day 7 p.i.) active foetal infection has ceased (Figure 4.3). All uninfected foetuses at that time were, presumably, passively immunised against infection. The still births associated with RRV infection may have occurred because of the apparent inability of maternal IgG to mediate recovery from established foetal infection. If, occasionally, foetal infection established just prior to the transfer of maternal IgG, a slower spread of virus through the foetal tissues may have resulted. Thus, although death of the foetus may have been inevitable, its survival may have been prolonged until parturition. It is difficult to attribute the enhanced post-natal death rate to the direct effect of virus replication because RRV could not be isolated from these neonates. Any adverse effect of the infection in the mother involving, for example, a reduced milk supply, may also have contributed to a failure of the neonates to thrive.

The observation that foetal infection with SFV could be blocked by the administration of specific immune ascites fluid to pre-infected mothers (Table 5.8) suggests that the outcome of this infection during pregnancy may be dependent on mechanisms similar to those proposed for RRV. Abortion may occur when virus migrates into all foetuses within a pregnancy before passive immunisation commences. This is in accordance with the conclusion made in Chapter 4 that the essential difference between the outcome of maternal infection with RRV and SFV may be related to an apparent greater efficiency of SFV in growing through the placenta into the foetal circulation. The fact that the immune ascites fluid prevented abortion but not placental infection in 3 animals also supports the proposal from Chapter 4 that abortion occurs as a consequence of foetal infection rather than any virus-induced impairment of placental or maternal function.

If the outcome of both in utero infections is dependent on the timing of virus growth through the placenta relative to the time of passive antibody transfer to the foetus, why is SFV more efficient at causing foetal infection than RRV? Because both viruses appear to require a similar time after maternal inoculation before foetal infection commences (Figures 4.3 and 4.7), the answer to this problem must be that effective foetal immunisation against SFV takes a longer time to achieve than against RRV. One possible reason for this is that although specific maternal IgG may be produced at the same time against each virus, the extensive areas of infected cells in the labrynth observed following SFV, but not RRV, infection (see Chapter 4) may adsorb the anti-viral antibody from the foetal circulation.

The results of this study indicate that the outcome of in utero infection with RRV or SFV depended on the timing of maternal inoculation with regard to the stage of gestation (Figures 4.9 and 4.11). Although this has been observed in other infections, it is

usually because either the foetus gains immuno-competence during later gestation, or the specific target cells or organs in the foetus may only be present or susceptible for a brief period of time (Kilham et al. 1967; Osburn et al. 1971a, 1971b; Parsonson et al. 1977). In the present study, however, the fact that neonatal mice are uniformly killed by both strains of virus suggests that foetal mice may be highly susceptible to infection throughout the pregnancy. It is possible that the observed variation in severity of the in utero infections may be more a function of placental development. In the mouse, blastocyst implantation occurs at 4.5 days after conception, and is complete at gestation day 6 (Rugh 1968). Perhaps the viruses are not able to grow through the immature placenta as efficiently as the day 10 or day 11 placenta? If so, this would allow more time for passive immunisation of the foetus to occur.

In conclusion, it appears that the pathogenesis and outcome of in utero infections of mice with RRV and SFV are dependent on similar mechanisms, which probably occur in both syngeneic and allogeneic pregnancies. The outcome of maternal infection with either virus appears to depend, in effect, on a "race" between protective maternal IgG and the virus for the foetus. When the antibody "wins", the foetus is protected, but when virus reaches the foetus first, death ensues. Because high titres of virus are present in each placenta it seems likely, but could not be proved, that virus will eventually cross into every foetus. It would therefore be of interest to examine the post-natal immune reactivity of the spared foetuses to see whether immune tolerance to the specific virus resulted.

The concept of the placenta as a total barrier to the passage of virus to the foetus, at least with the infections in the present study, does not appear to be viable. Nevertheless, the ability of the placenta to delay the passage of RRV to the foetus by 1 or 2 days is of vital importance to the pregnancy in that it allows sufficient time for the passive

immunisation of those foetuses not already infected. This is not the case with SFV, because, for unknown reasons, adequate immunisation of the foetus appears to take longer than with RRV. These results support Fox (1977) who suggested that any resistance offered by the placenta to the passage of virus to the foetus is not absolute but only delaying, and is largely due to it acting in a non-specific fashion as a physical barrier.

CHAPTER 6

THE ROLE OF COLOSTRUM AND MILK IN PROTECTION OF THE NEONATAL MOUSE AGAINST PERIPHERAL INFECTION WITH ROSS RIVER VIRUS.

INTRODUCTION

The results in Chapter 5 indicate that passively acquired maternal antibody may protect a proportion of foetuses within a pregnancy from *in utero* infection with RRV. The mechanisms involved in the pre-natal antibody transfer were highly selective for the 4 maternal IgG subclasses. It was considered that the timing of antibody transfer from the mother relative to the time of virus growth through the placenta was of critical importance and determined whether or not a particular foetus was spared. As was indicated in Chapter 1.3, however, maternal antibody passively acquired pre-natally may not be as important as that acquired post-natally for the protection of the mouse neonate against infection. The experiments described in this Chapter were therefore designed to examine whether colostrum and milk from an immune mouse could protect susceptible neonates against peripheral challenge with RRV.

RESULTS

Does colostrum and milk from an immune mother protect a neonatal mouse against i.p. infection with RRV?

To assess the ability of colostrum and milk from an immune mother to protect a neonate against RRV infection, it was necessary to ensure that any antibody acquired by the neonate before birth did not influence its immune status. Thus, newborn mice from uninfected mothers were fostered onto post-parturient mothers that had been infected i.p. with 2600 pfu of RRV at gestation day 11. Ten control neonates, suckling on their own uninfected mothers, and 10 of the neonates fostered onto immune mothers, were challenged i.p. with 1000 pfu of RRV 9 days after birth. The results (Table 6.1) show that immunity against challenge was conferred by suckling on the immune mother.

Table 6.1 The effect of the ingestion of immune colostrum and milk on the susceptibility of neonates[a] to i.p. infection with RRV

Mothers[b]	Number of challenged neonates[c]	Number surviving challenge	Mean survival time (days)
Immune	10	10	-
Non-immune	10	0	6.2

a All neonates were from uninfected mothers.

b Immune mothers had been infected i.p. with 2600 pfu of RRV at gestation day 11. Neonates were fostered immediately after parturition.

c All neonates were challenged i.p. with 1000 pfu of RRV at the 9th day of life.

The nature of the protection

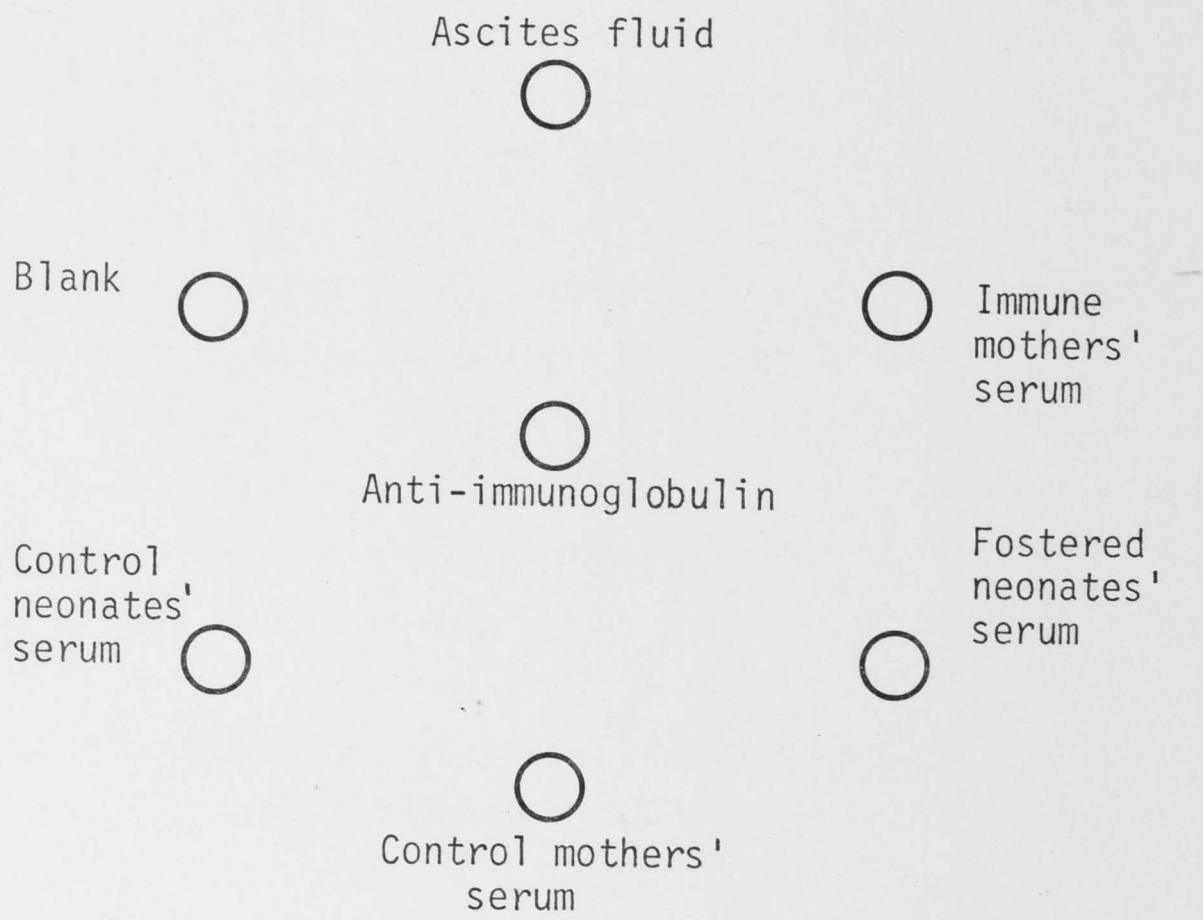
An experiment was designed to examine whether protective antibody was present in the serum of the neonates suckling on immune mothers and, if so, which antibody classes could be detected.

A group of 30 neonates from uninfected mothers were fostered onto 5 post-parturient mothers that had been infected i.p. with 2600 pfu of RRV at gestation day 11. Nine days after fostering, both groups were killed and their serum pooled. Serum pools from 5 uninfected mothers and their 32 neonates at 9 days of age were used as controls. The immunoglobulins present in these 4 pools, and in hyper-immune ascites fluid made against RRV as a known high-titred antiserum, were identified by the gel diffusion test (Figure 6.1, Table 6.2). All 6 immunoglobulin isotypes were detected in the ascites fluid and the 2 maternal serum pools. In the neonatal sera, IgM and IgA were not detectable, with precipitin lines only being observed for the IgG subclasses.

All 4 serum pools were fractionated on a Sepharose CL-4B-protein A column. The amount of serum protein loaded onto the column was: 71.7 mg of immune mothers' serum; 89.6 mg of control mothers' serum; 42.2 mg of fostered neonates' serum; and 82.9 mg of control neonates' serum. The optical density of each fraction in the Coomassie Blue reaction is presented in Figure 6.2, and is proportional to the protein content. Because the elution pattern obtained with the hyper-immune ascites fluid (Figure 5.2) indicated that 4 major protein peaks were present, for each of the other serum pools the fractions in the vicinity of these peaks were pooled, concentrated, and the immunoglobulin subclasses identified (Table 6.2). The locations of the different immunoglobulins were: peak 1, IgA and IgM; peak 2, IgG1; peak 3, IgG2a and IgG3; peak 4, IgG2b. All isotypes detected in the non-fractionated serum pools were also

Figure 6.1 Gel diffusion test: The identity of the immunoglobulin isotypes present in non-fractionated serum pools

All tests were set up as follows:



Anti-immunoglobulins were:

Test A = Anti-IgM

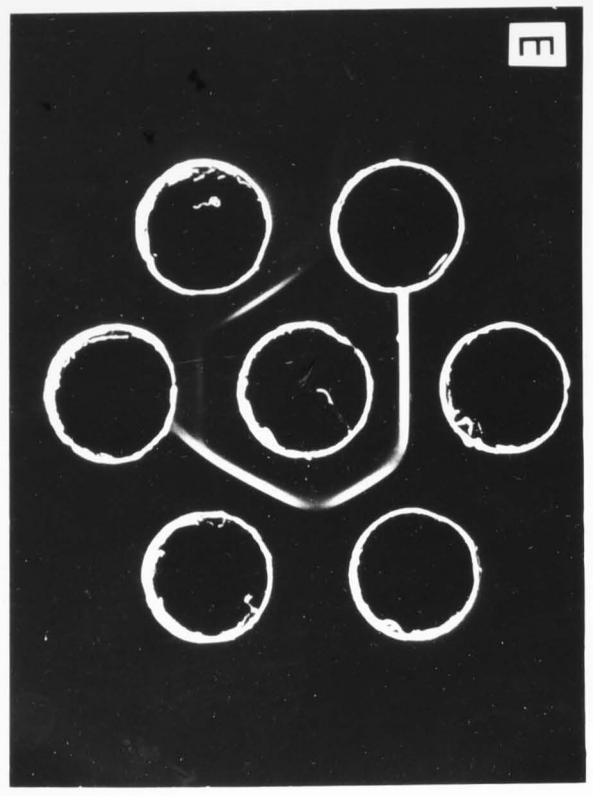
Test B = Anti-IgA

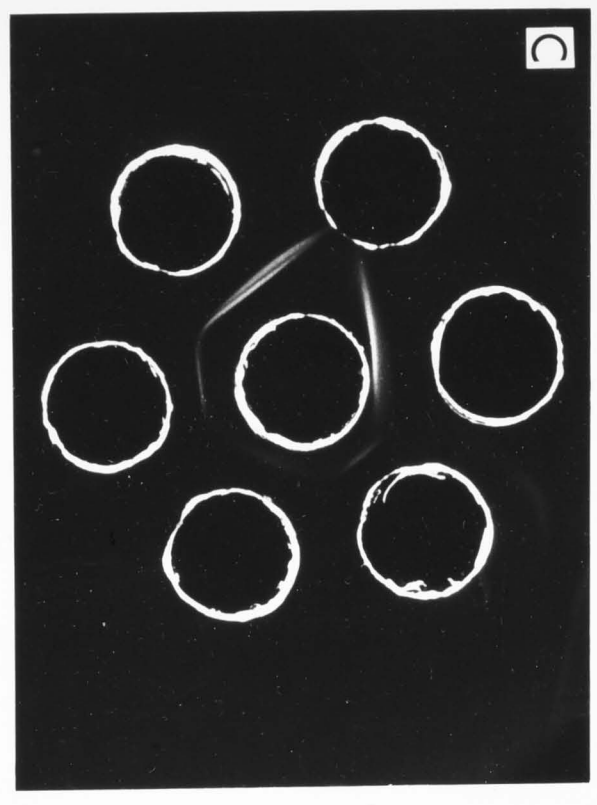
Test C = Anti-IgG1

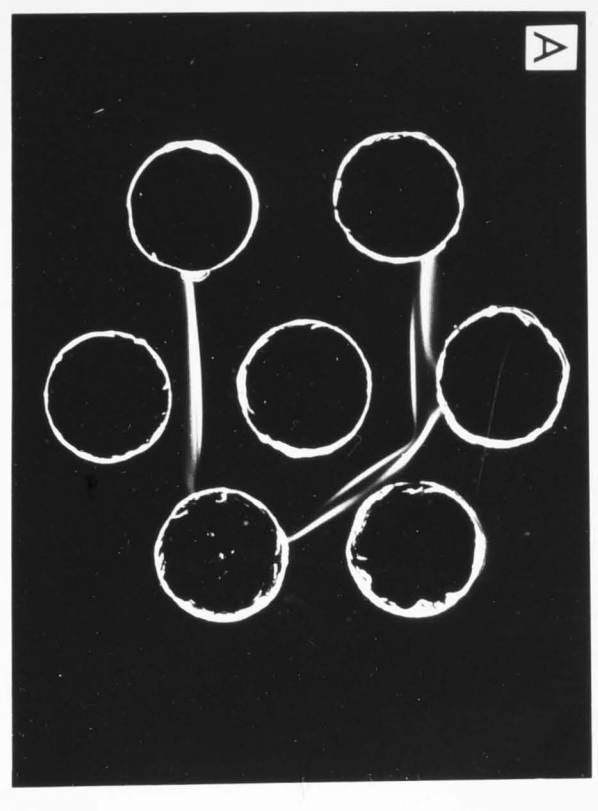
Test D = Anti-IgG2a

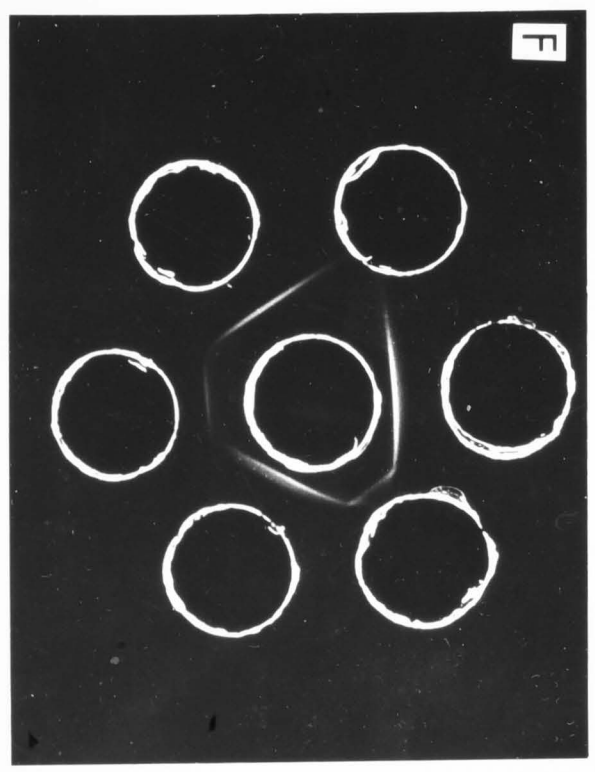
Test E = Anti-IgG2b

Test F = Anti-IgG3


E


C


A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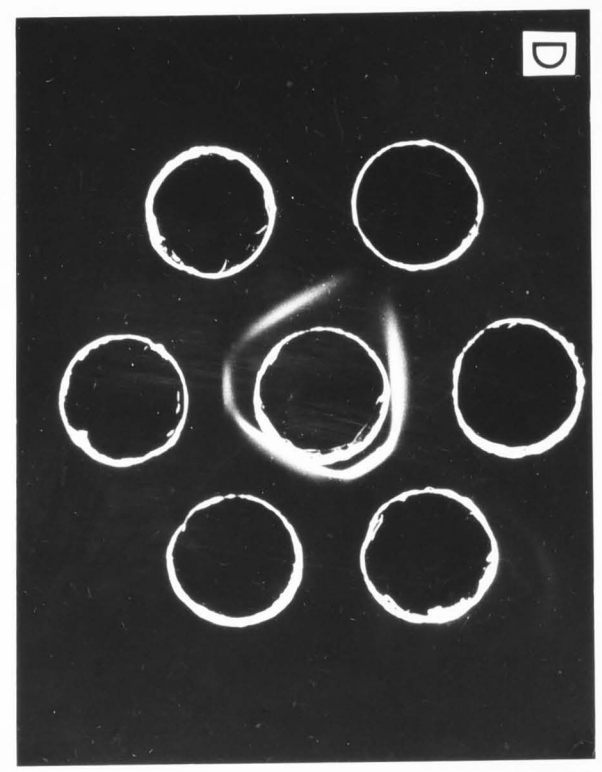

D

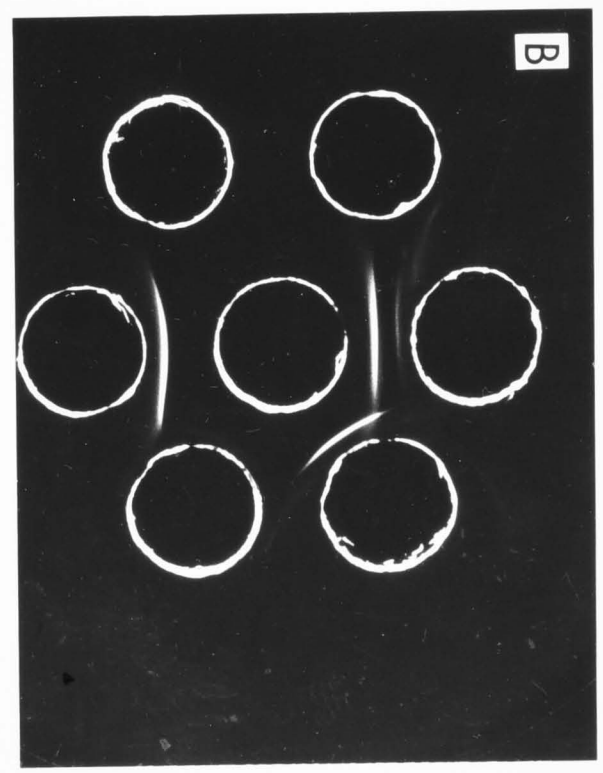

B

Table 6.2 The identity of immunoglobulins detected in various fractions[a] of maternal and neonatal sera

Peak Number	Fractions[b] (approx.)	Antibody detected[c]				
		Ascites fluid	Immune[d] Mother	Fostered[e] neonate	Control[f] mother	Control[f] neonate
1	1 - 17	A, M	A, M	-	A, M	-
2	20 - 40	M, G1	G1	G1	G1	G1
3	44 - 54	M, G2a, G3	G2a, G3	G2a, G3	G2a, G3	G2a, G3
4	57 on	G2b	G2b	G2b	G2b	G2b
Non-fractionated serum		A, M, G1, G2a, G2b, G3	A, M, G1, G2a, G2b, G3	G1, G2a, G2b, G3	A, M, G1, G2a, G2b, G3	G1, G2a, G2b, G3

a Sera were fractionated on a Sepharose CL-4B-protein A column using a linear pH gradient. The elution pattern for the Ascites fluid is presented in Figure 5.2, and the patterns for the other serum pools are presented in Figure 6.2

b Peak sizes varied slightly between sera but always encompassed the relevant fractions.

c In the gel diffusion test. Immunoglobulin class or subclass presented.

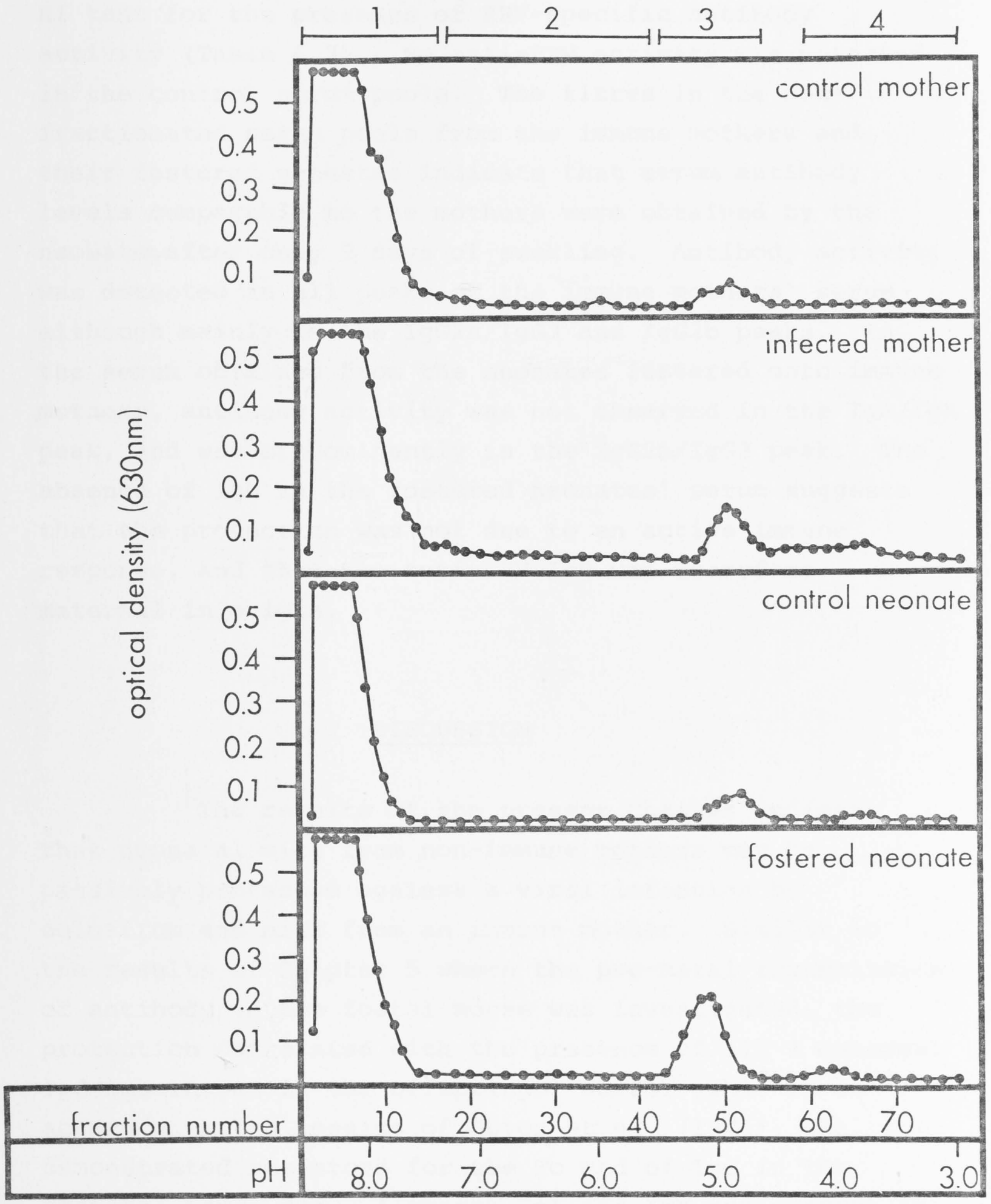
d Mothers were immunised by i.p. challenge with 2600 pfu of RRV at gestation day 11.

e Neonates from uninfected mothers were fostered onto post-parturient immune mothers. Serum was taken from the mothers and their neonates 9 days after birth.

f Control sera were from uninfected mothers and their neonates taken 9 days after birth.

Figure 6.2 Elution patterns obtained with various serum samples after fractionation on a Sepharose CL-4B-protein A column.

Newborn mice from uninfected mothers were fostered onto mothers that had been infected i.p. with 2600 pfu of RRV at gestation day 11, and had just given birth. Controls were uninfected mothers and their neonates. Serum samples were taken from mothers and neonates 9 days after birth. The optical density of each fraction after treatment with Coomassie Blue is plotted, and is proportional to the protein content. The approximate positions of the 4 major peaks detected in the hyper-immune fluid (presented in Figure 5.1) are shown at the top of the figure.


1
2
3
4
control mother
infected mother
control neonate
fostered neonate
optical density (630nm)
0.5
0.4
0.3
0.2
0.1
fraction number
10
20
30
40
50
60
70
pH
8.0
7.0
6.0
5.0
4.0
3.0

detected in the fractionated peaks.

All serum pools and peaks were tested in the HI test for the presence of RRV-specific antibody activity (Table 6.3). No anti-RRV activity was detected in the control serum pools. The titres in the non-fractionated serum pools from the immune mothers and their fostered neonates indicate that serum antibody levels comparable to the mothers were obtained by the neonates after only 9 days of suckling. Antibody activity was detected in all peaks of the immune mothers' serum although mainly in the IgG2a/IgG3 and IgG2b peaks. In the serum obtained from the neonates fostered onto immune mothers, antibody activity was not observed in the IgM/IgA peak, and was predominantly in the IgG2a/IgG3 peak. The absence of IgM in the fostered neonates' serum suggests that the protection was not due to an active immune response, and that the anti-RRV IgG was therefore maternal in origin.

DISCUSSION

The results of the present Chapter indicate that neonatal mice from non-immune mothers may be passively protected against a viral infection by colostrum and milk from an immune mother. Similar to the results of Chapter 5 where the pre-natal transmission of antibody to the foetal mouse was investigated, the protection correlated with the presence of all 4 maternal IgG subclasses in the offsprings' serum. This is in accord with the results of Guyer et al. (1976), who demonstrated receptors for the Fc end of IgG in the neonatal mouse gut. Although IgA and IgM are present in mouse colostrum (Guyer et al. 1976), in the present study they were not absorbed by the neonate in detectable quantities. This agrees with Fahey and Barth (1965) and Iida et al. (1973), and suggests that the major role of colostral IgA and IgM is in defense of the neonate against enteric pathogens.

Table 6.3 HI antibody in peaks[a] from immune and control mothers[b] and their neonates

Peak Number	HI titre[c]			
	Immune mother	Fostered[d] neonate	Control	
			Mother	Neonate
1	10	< 10	< 10	< 10
2	2	32	< 2	< 2
3	256	2048	< 2	< 2
4	128	8	< 2	< 2
Non-fractionated serum	640	1280	< 10	< 10

a Peaks correspond to those in Figure 6.2 and Table 6.2

b Mothers were immunised by i.p. challenge with 2600 pfu of RRV at gestation day 11. Control mothers were uninfected.

c Reciprocal of the HI titre per ml.

d Neonatal mice from uninfected mothers were fostered onto post-parturient immune mothers. Serum was taken 9 days after fostering.

The duration of the immunity conferred on the neonate post-natally could not be established because mice become resistant to the lethal effects of RRV infection at approximately 3 weeks of age. Nevertheless, the observation that, in the mouse, antibody can be absorbed from milk for up to 17 days after birth (Hemmings and Morris 1959), suggests that the post-natal acquisition of passive immunity may have provided a longer term protection against RRV infection than that acquired *in utero*.

There was a marked difference between the relative concentrations of the IgG subclasses in neonatal serum compared to maternal serum (Table 6.3). This may be because the affinity of the intestinal receptors for IgG in the neonatal mouse varies for each subclass, with the highest affinity being observed for IgG2a and IgG1 (Guyer *et al*. 1976). Unfortunately, the relative contributions of IgG2a and IgG3 to the total neonatal serum antibody could not be differentiated using the Sepharose-protein A column. Nevertheless, the observations of Guyer *et al*. (1976) that IgG2a is the major maternal serum IgG subclass in the mouse, and that IgG3 is, at least in the absence of infection, only present in trace amounts in colostrum, suggests that IgG2a is probably the predominant antibody in the IgG2a/IgG3 peak.

CHAPTER 7

GENERAL DISCUSSION

Infections which influence the establishment and continuation of a healthy pregnancy, and the subsequent well-being of the young, are of considerable interest and importance in both human and veterinary medicine. In human medicine the social and humanitarian consequences of these infections have received greatest consideration whilst in veterinary medicine the effects on animal productivity and economics are the most important features. However, although the consequences of infection may be important in man and animals for different reasons, an understanding of pathological processes in one species is frequently of importance in solving similar problems in another (Coid 1977). Therefore, in the present study, rather than attempting to obtain information about a specific pathogen of importance to either human or veterinary medicine, it was decided to establish a laboratory model to try to obtain a more general idea of the mechanisms which may occur in in utero virus infections. The laboratory mouse was chosen as the model because of its ease of breeding, storage and handling, the availability of large numbers of mice of different inbred strains, its short gestation period, and the ability to obtain pregnant mice at specified times of gestation. Because experiments using pregnant animals can take considerably more time than those with non-pregnant animals, in that once an adequate number have been mated they might not be used until days or weeks later, it was considered that the use of the mouse provided an opportunity to perform more intensive investigations than larger animals. Indeed, the mouse has been used as a model of numerous viral infections during pregnancy, some of which include reovirus type 2 (Hashimi et al. 1966); CMV (Johnson 1969); St. Louis encephalitis virus (Anderson and Hanson 1970); coxsackievirus B3 (Lansdown and Coid 1974; Lansdown 1975); polyoma virus (McCance and Mims 1977); Ross River virus, getah and Murray Valley encephalitis virus (Aaskov et al. 1981a); and Japanese encephalitis virus (Mathur et al. 1981).

Whilst the size, gestation period, type of placentation, physiology and endocrinology of the mouse differs from other species, there are some points raised in the present study that may be applicable to *in utero* infections in general. In particular, I would like to emphasize 2 of these. Firstly, the hypothesis that the outcome of *in utero* infection in the mouse depends on the relative timing of virus growth through the placenta with regard to the timing of the transfer of passive immunity from the mother may be applicable to all species that have a significant pre-natal transfer of antibody to the foetus. In fact, cases of placental infection without foetal infection would be more logically explained by this hypothesis than the concept of the placenta acting as a total barrier to the passage of virus to the foetus. This is especially so in light of the observation that virus is able to persist in the trophoblast in the face of a maternal immune response. Thus, the isolation of virus from placental tissue but not the foetus in human infections with rubella (Alford *et al*. 1964) and CMV (Hayes and Gibas 1971) could be because the foetuses were passively immunised before virus was able to grow through the placenta. It is of interest that Cooper *et al*. (1969), reporting on the unpredictability of *in utero* infections, cited a case of non-identical twins whose mother had rubella during pregnancy, where only one child displayed the congenital syndrome. It is tempting to speculate that, similar to the situation with *in utero* infections with RRV in the present study, specific maternal IgG was transferred to the spared foetus in time to be protective but was too late to prevent infection in the other.

The second point concerns the expression of maternal immunity in the foeto-placental unit. The results of the present study indicate that virus present in placental tissue is not eliminated by the maternal cellular or humoral immune responses. This suggests that viral antigen (in association with MHC antigens) is either blocked, not expressed, or not recognized.

This could be because the infected trophoblast observed within the body of the placenta is not accessible to the maternal leukocytes. It is also possible that the expression of the immune response is suppressed in the micro-environment of the placenta. If this latter is the case, the mechanisms involved in the immune suppression may be linked to one of the fundamental questions of reproductive and transplantation biology - why is a foetus which displays paternal alloantigens not rejected by the mother? Therefore, investigations as to whether anti-viral cellular immunity is functionally expressed against infected trophoblast may provide another avenue for the elucidation of this problem.

CHAPTER 8

BIBLIOGRAPHY

AASKOV, J.G., DAVIES, C.E.A., TUCKER, M. and DALGLISH, D. (1981a). Effect on mice of infection during pregnancy with three Australian arboviruses. Am. J. Trop. Med. Hyg. 30, 198.

AASKOV, J.G., NAIR, K., LAWRENCE, G.W., DALGLISH, D.A. and TUCKER, M. (1981b). Evidence for trans-placental transmission of Ross River virus in humans. Med. J. Aust. 2, 20.

ALBERMAN, E. and PECKHAM, C. (1977). Long-term effects following infections in pregnancy. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. 489.

ALFORD, C.A. (1965). Studies on antibody in congenital rubella infections. Am. J. Dis. Child. 110, 455.

ALFORD, C.A., NEVA, F.A. and WELLER, T.H. (1964). Virologic and serologic studies on human products of conception after maternal rubella. N. Engl. J. Med. 271, 1275.

AL-NAKIB, W., BEST, J.M. and BANATVALA, J.E. (1975). Rubella-specific serum and nasopharyngeal immunoglobulin responses following naturally acquired and vaccine-induced infection. Prolonged persistence of virus-specific IgM. Lancet i, 182.

ANDERSON, D.J. (1978). The responsiveness of various maternal mouse lymphocyte populations to mitogenic stimulation in vitro. Cell. Immunol. 41, 150.

ANDERSON, A.A. and HANSON, R.P. (1970). Experimental transplacental transmission of St. Louis encephalitis virus in mice. Infect. Immun. 2, 320.

ARTHUR, G.H. (1975). In "Veterinary Reproduction and Obstetrics". 4th edition. Bailliere Tindall. p. 106.

BANATVALA, J.E. (1977). Health of mother, fetus and neonate following maternal viral infections during pregnancy. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. 437.

BANATVALA, J.E., HORSTMANN, D.M., PAYNE, M.C. and GLUCK, L. (1965). Rubella syndrome and thrombocytopenic purpura in newborn infants. Clinical and virologic observations. N. Engl. J. Med. 273, 474.

BANATVALA, J.E., POTTER, J.E. and BEST, J.M. (1971). Interferon response to Sendai and rubella viruses in human foetal cultures, leucocytes and placental cultures. J. Gen. Virol. 13, 193.

BARKER, C.F. and BILLINGHAM, R.E. (1968). The role of afferent lymphatics in the rejection of skin homografts. J. Exp. Med. 128, 197.

BARLOW, R.M., VANTSIS, J.T., GARDINER, A.C., RENNIE, J.C., HERRING, J.A. and SCOTT, F.M.M. (1980). Mechanisms of natural transmission of border disease. J. Comp. Pathol. 90, 57.

BEER, A.E. and BILLINGHAM, R.E. (1970). Implantation, transplantation and epithelial-mesenchymal relationships in the rat uterus. J. Exp. Med. 132, 721.

BEER, A.E. and BILLINGHAM, R.E. (1971). Immunobiology of mammalian reproduction. Adv. Immunol. 14, 1.

BEER, A.E. and BILLINGHAM, R.E. (1974). Host responses to intra-uterine tissue, cellular and fetal allografts. J. Reprod. Fertil. 21, 59.

BEER, A.E. and BILLINGHAM, R.E. (1978). Immunoregulatory aspects of pregnancy. Fed. Proc. 37, 2374.

BEER, A.E. and BILLINGHAM, R.E. (1979). Maternal immunological recognition mechanisms during pregnancy. In "Maternal recognition of pregnancy". Ciba Foundation 64. Excerpta Medica. p. 293.

BEER, A.E., BILLINGHAM, R.E. and HOERR, R.A. (1971). Elicitation and expression of transplantation immunity in the uterus. Transplant. Proc. 3, 609.

BELL, S.C. and BILLINGTON, W.D. (1980). Major anti-paternal alloantibody induced by murine pregnancy is non-complement-fixing IgG1. Nature 288, 387.

BELL, S.C. and BILLINGTON, W.D. (1981). Humoral immune responses in murine pregnancy. I. Anti-paternal alloantibody levels in maternal serum. J. Reprod. Immunol. 3, 3.

BENTVELZEN, P., DAAMS, J.H., HAGEMAN, P. and CALAFAT, J. (1970). Genetic transmission of viruses that incite mammary tumor in mice. Proc. Natl. Acad. Sci. U.S.A. 67, 377.

BERGER, M.L. and BLANDEN, R.V. (1981). The T-lymphocyte in infectious pathology. Pathol. Res. Pract. 171, 128.

BIGGERS, J.D. (1980). Fetal and neonatal physiology. In "Medical Physiology". 14th edition, Vol. 2. Ed. V.B. Mountcastle. C.V. Mosby. p. 1947.

BILLINGHAM, R.E. (1964). Transplantation immunity and the maternal-fetal relation. N. Engl. J. Med. 270, 667.

BILLINGTON, D. (1975). Organisation, ultrastructure and histochemistry of the placenta: immunological considerations. In "Immunobiology of trophoblast". Eds. R.G. Edwards, C.W.S. Howe and M.H. Johnson. Cambridge University Press. p. 67.

BIRKELAND, S.A., TEISNER, B., SCHILLING, W., KEMP, E., PEDERSEN, G.T., and SVEHAG, S-E. (1979). Effect of pregnancy zone protein on leukocyte migration inhibition, lymphocyte transformation and rosette formation by lymphocytes. Acta. Pathol. Microbiol. Scand. (C) 87, 235.

BISHOP, D.H.L., and SHOPE, R.E. (1980). Bunyaviridae. Compr. Virol. 14, 1.

BJORKSTEN, B. (1980). Phagocyte function in pregnancy. Immunol. Today 1, 55.

BJORKSTEN, B., SODERSTROM, T., DAMBER, M-G., VON SCHOULTZ, B. and STIGBRAND, T. (1978). Polymorphonuclear leucocyte function during pregnancy. Scand. J. Immunol. 8, 257.

BLATTNER, R.J. (1974). The role of viruses in congenital defects. Am. J. Dis. Child. 128, 781.

BLITHEL, J.F., DRAPER, G.J. and GORBACH, P.D. (1973). Association between malignant disease in children and maternal virus infections. Br. Med. J. i, 706.

BLOT, W.J., DRAPER, G., KINLEN, L. and WILSON, M.K. (1980). Childhood cancer in relation to prenatal exposure to chickenpox. Br. J. Cancer 42, 342.

BRADISH, C.J., FITZGEORGE, R., TITMUSS, D. and BASKERVILLE, A. (1979). The responses of nude-athymic mice to nominally avirulent Togavirus infections. J. Gen. Virol. 42, 555.

BRAMBELL, F.W.R. (1966). The transmission of immunity from mother to young and the catabolism of immunoglobulins. Lancet ii, 1087.

BRENIERE, S. and VIENS, P. (1980). Trypanosoma musculi: transfer of immunity from mother mice to litter. Can. J. Microbiol. 26, 1090.

BRUCE-CHWATT, L.J. and GIBSON, F.D. (1956). Trans-placental passage of Plasmodium berghei and passive transfer of immunity in rats and mice. Trans. R. Soc. Trop. Med. Hyg. 50, 47.

BUERGELT, C.D., HALL, C.E., MERKAL, R.S., WHITLOCK, R.H. and DUNCAN, J.R. (1977). Lymphocyte transformation: an aid in the diagnosis of paratuberculosis. Am. J. Vet. Res. 38, 1709.

BULLEN, J.J. (1981). The role of milk and gut flora in protection of the newborn against infection. In "Immunological aspects of infection in the fetus and newborn". Eds. H.P. Lambert and C.B.S. Wood. Academic Press. p. 123.

BURNET, F.M. and FENNER, F. (1949). Theoretical aspects of antibody production. In "The production of antibodies". 2nd edition. MacMillan. p. 78.

BUTLER, N.R., DUDGEON, J.A., HAYES, K., PECKHAM, C.S. and WYBAR, K. (1965). Persistence of rubella antibody with and without embryopathy. Br. Med. J. ii, 1027.

CAMPBELL, C.H. (1960). The susceptibility of mother mice and pregnant mice to the virus of foot-and-mouth disease. J. Immunol. 84, 469.

CANTOR, H. and BOYSE, E.A. (1975). Functional subclasses of T lymphocytes bearing different Ly antigens. J. Exp. Med. 141, 1376.

CARLSSON, B., CRUZ, J.R., MELLANDER, L. and HANSON, L.A. (1980). The mechanisms of immunity provided by breast feeding. In "Human milk: its biological and social value". Eds. S. Freier and A.I. Eidelman. Excerpta Medica. p. 122.

CARRETTI, N. and OVARY, Z. (1969). Transmission of γG antibodies from maternal to fetal circulation in the mouse. Proc. Soc. Exp. Biol. Med. 130, 509.

CATALANO, L.W., and SEVER, J.L. (1971). The role of viruses as causes of congenital defects. Annu. Rev. Microbiol. 25, 255.

CHAOUAT, G. and VOISIN, G.A. (1979). Regulatory T cell subpopulations in pregnancy. I. Evidence for suppressive activity of the early phase of MLR. J. Immunol. 122, 1383.

CHAOUAT, G., VOISIN, G.A., ESCALIER, D. and ROBERT, P. (1979). Facilitation reaction (enhancing antibodies and suppressor cells) and rejection reaction (sensitised cells) from the mother to the paternal antigens of the conceptus. Clin. Exp. Immunol. 35, 13.

CHATTERJEE-HASROUNI, S. and LALA, P.K. (1981). MHC antigens on mouse trophoblast cells: paucity of Ia antigens despite the presence of H-2K and D. J. Immunol. 127, 2070.

CHATTERJEE-HASROUNI, S. and LALA, P.K. (1982). Localisation of paternal H-2K antigens on murine trophoblast cells in vivo. J. Exp. Med. 155, 1679.

CHISCON, M.O. and GOLUB, E.S. (1972). Functional development of the interacting cells in the immune response. I. Development of T cell and B cell function. J. Immunol. 108, 1379.

CLARK, D.A. and McDERMOTT, M.R. (1978). Impairment of host versus graft reaction in pregnant mice. I. Suppression of cytotoxic T cell generation in lymph nodes draining the uterus. J. Immunol. 121, 1389.

CLARK, D.A. and McDERMOTT, M.R. (1981). Active suppression of host-versus-graft reaction in pregnant mice. III. Developmental kinetics, properties and mechanism of induction of suppressor cells during first pregnancy. J. Immunol. 127, 1267.

CLARK, D.A., McDERMOTT, M.R. and SZEWCZUK, M.R. (1980). Impairment of host-versus-graft reaction in pregnant mice. II. Selective suppression of cytotoxic T cell generation correlates with soluble suppressor activity and with successful allogeneic pregnancy. Cell. Immunol. 52, 106.

CLARKE, D.H. and CASALS, J. (1958). Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg. 7, 561.

COID, C.R. (1977). In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. vii.

COOPER, L.Z., FEDUN, B.A., MATTERS, B.A. and KRUGMAN, S. (1969). Maternal rubella and the risk to the fetus. In "Symposia series in immunobiological standardisation, volume 11. International symposium on rubella vaccines". S. Karger. p.73.

CRADOCK-WATSON, J.E., RIDEHALGH, M.K.S., ANDERSON, M.J., PATTISON, J.R. and KANGRO, H.O. (1980). Fetal infection resulting from maternal rubella after the first trimester of pregnancy. J. Hyg. Camb. 85, 381.

CURRIE, G.A., van DOORNINCK, W. and BAGSHAWE, K.D. (1968). Effect of neuraminidase on the immunogenicity of early mouse trophoblast. Nature 219, 191.

CURZEN, P., JONES, E. and GAUGAS, J. (1972). Immunological responses in pregnancy. Br. Med. J. iv, 49.

DALLDORF, G. and GIFFORD, R. (1954). Susceptibility of gravid mice to coxsackie virus infection. J. Exp. Med. 99, 21.

DALMASSO, A.P., MARTINEZ, C., SJODIN, K. and GOOD, R.A. (1963). Studies in the role of the thymus in immunobiology. J. Exp. Med. 118, 1089.

DAVIDSON, W.F. and PARISH, C.R. (1975). A procedure for removing red cells and dead cells from lymphoid cell suspensions. J. Immunol. Methods 7, 291.

DIAZ-JOUANEN, E. and WILLIAMS, R.C. (1974). T and B lymphocytes in human colostrum. Clin. Immunol. Immunopath. 3, 248.

DOHERTY, P.C. and BENNINK, J.R. (1981). Monitoring the integrity of self: biology of MHC-restriction of virus-immune T cells. Fed. Proc. 40, 218.

DONE, J.T., TERLECKI, S., RICHARDSON, C., HARKNESS, J.W., SANDS, J.J., PATTERSON, D.S.P., SWEASEY, D., SHAW, I.G., WINKLER, C.E. and DUFFELL, S.J. (1980). Bovine virus diarrhoea-mucosal disease virus: pathogenicity for the foetal calf following maternal inoculation. Vet. Rec. 106, 473.

DUDGEON, J.A. (1969). Congenital rubella. Pathogenesis and immunology. Am. J. Dis. Child. 118, 35.

EBBIN, A.J., WILSON, M.G., WEHRLE, P.F., CHIN, J., EMMONS, R.W. and LENNETTE, E.H. (1972). Rubella vaccination and pregnancy. Lancet ii, 481.

ELLIS, P.R. and HUGH-JONES, M.E. (1977). Economics of infection during pregnancy in farm livestock. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. 565.

EMBIL, J.A., MANUEL, F.R., GARNER, J.B. and COVENEY, L. (1982). Cytomegalovirus in the semen. J. Can. Med. Assoc. 126, 391.

ENDERS, A.C. (1965). A comparative study of the fine structure of the trophoblast in several hemochorial placentas. Am. J. Anat. 116, 29.

EY, P.L. (1973). Ph.D. thesis, Australian National University. p. 32.

EY, P.L., PROWSE, S.J. and JENKIN, C.R. (1978). Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using protein A-sepharose. Immunochemistry 15, 429.

FAHEY, J.L. and ROBINSON, A.G. (1963). Factors controlling serum γ-globulin concentration. J. Exp. Med. 118, 845.

FAHEY, J.L. and BARTH, W.F. (1965). The immunoglobulins of mice. 4. Serum immunoglobulin changes following birth. Proc. Soc. Exp. Biol. Med. 118, 596.

FARBER, P.A. and GLASGOW, L.A. (1968). Factors modifying host resistance to virus infection. II. Enhanced susceptibility of mice to encephalomyocarditis virus infection during pregnancy. Am. J. Pathol. 53, 463.

FAULK, W.P. and TEMPLE, A. (1976). Distribution of β2 microglobulin and HLA in chorionic villi of human placentae. Nature 262, 799.

FAULK, W.P. and McINTYRE, J.A. (1981). Trophoblast survival. Transplantation 32, 1.

FAULK, W.P., SANDERSON, A.R. and TEMPLE, A. (1977). Distribution of MHC antigens in human placental chorionic villi. Transplant. Proc. 9, 1379.

FEDRICK, J. and ALBERMAN, E.D. (1972). Reported influenza in pregnancy and subsequent cancer in the child. Br. Med. J. ii, 485.

FIELD, P.R., SHANKER, S. and MURPHY, A.M. (1980). The use of protein A-sepharose affinity chromatography for separation and detection of specific IgM antibody in acquired rubella infection: A comparison with absorption by Staphylococci containing protein A and density gradient ultracentrifugation. J. Immunol. Meth. 32, 59.

FISHAUT, M., MURPHY, D., NEIFERT, M., McINTOSH, K. and OGRA, P.L. (1981). Bronchomammary axis in the immune response to respiratory syncytial virus. J. Pediatr. 99, 186.

FLORMAN, A.L., GERSHON, A.A., BLACKETT, P.R. and NAHMIAS, A.J. (1973). Intrauterine infection with herpes simplex virus. Resultant congenital malformations. J. Am. Med. Assoc. 225, 129.

FORREST, J.M. and MENSER, M.A. (1974). Gammaglobulin and congenital rubella. Br. Med. J. ii, 439.

FORSGREN, A. and SJOQUIST, J. (1966). "Protein A" from S. aureus. J. Immunol. 97, 822.

FOWLER, A.K., REED, C.D. and GIRON, D.J. (1980). Identification of an interferon in murine placentas. Nature 286, 266.

FOX, H. (1977). Infections of the placenta. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p.251.

FOX, H. (1981). Invited review. A contemporary approach to placental pathology. Pathology 13, 207.

FUCCILLO, D.A. and SEVER, J.L. (1973). Viral teratology. Bacteriol. Rev. 37, 19.

FUCCILLO, D.A., STEELE, R.W., HENSEN, S.A., VINCENT, M.M., HARDY, J.B. and BELLANTI, J.A. (1974). Impaired cellular immunity to rubella virus in congenital rubella. Infect. Immun. 9, 81.

GAMSU, H. (1977). Health of mother, fetus and neonate following bacterial, fungal and protozoal infections during pregnancy. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p.343.

GARDINER, A.C. and BARLOW, R.M. (1981). Vertical transmission of border disease infection. J. Comp. Pathol. 91, 467.

GERSHON, A.A. (1975). Varicella in mother and infant: problems old and new. Prog. Clin. Biol. Res. 3, 79.

GIBBS, E.P.J., LAWMAN, M.J.P. and HERNIMAN, K.A.J. (1979). Preliminary observations on transplacental infection of blue tongue virus in sheep - a possible over wintering mechanism. Res. Vet. Sci. 27, 118.

GOODFELLOW, P.N., BARNSTABLE, C.J., BODMER, W.F., SNARY, D. and CRUMPTON, M.J. (1976). Expression of HLA system antigens on placenta. Transplantation 22, 595.

GREGG, N.McA. (1941). Congenital cataract following German measles in the mother. Trans. Ophthalmol. Soc. Aust. 3, 35.

GREY, H.M., HIRST, J.W., COHN, M. (1971). A new mouse immunoglobulin: IgG3. J. Exp. Med. 133, 289.

GRIFFIN, D.E. and JOHNSON, R.T. (1973). Cellular immune response to viral infection: In vitro studies of lymphocytes from mice infected with sindbis virus. Cell. Immunol. 9, 426.

GROSS, L. (1951). Pathogenic properties and "vertical" transmission of the mouse leukemia agent. Proc. Soc. Exp. Biol. Med. 78, 342.

GUYER, R.L., KOSHLAND, M.E. and KNOPF, P.M. (1976). Immunoglobulin binding by mouse intestinal epithelial cell receptors. J. Immunol. 117, 587.

HAGEN, C. and FROLAND, A. (1972). Depressed lymphocyte response to P.H.A. in women taking oral contraceptives. Lancet i, 1185.

HALLIDAY, R. (1956). The termination of the capacity of young rats to absorb antibody from the milk. Proc. R. Soc. Lond. Ser. B. 145, 179.

HAMILTON, M.S. and HELLSTROM, I. (1977). Altered immune responses in pregnant mice. Transplantation 23, 423.

HANSHAW, J.B. (1971). Congenital cytomegalovirus infection: a fifteen year perspective. J. Infect. Dis. 123, 555.

HARDY, J.B., McCRACKEN, G.H., GILKESON, M.R. and SEVER, J.L. (1969). Adverse fetal outcome following maternal rubella after the first trimester of pregnancy. J. Am. Med. Assoc. 207, 2414.

HARRISON, M.R. (1976). Maternal immunocompetence. II. Proliferative responses of maternal lymphocytes in vitro and inhibition by serum from pregnant rats. Scand. J. Immunol. 5, 881.

HASHIMI, A., CARRUTHERS, M.M., WOLF, P. and LERNER, A.M. (1966). Congenital infections with reovirus. J. Exp. Med. 124, 33.

HAYES, K. and GIBAS, H. (1971). Placental cytomegalovirus infection without fetal involvement following primary infection in pregnancy. J. Pediatr. 79, 401.

HELLSTROM, K.E., HELLSTROM, I. and BRAWN, J. (1969). Abrogation of cellular immunity to antigenically foreign mouse embryonic cells by a serum factor. Nature 224, 914.

HEMMINGS, W.A. and MORRIS, I.G. (1959). An attempt to affect the selective absorbtion of antibodies from the gut in young mice. Proc. R. Soc. Lond. Ser. B. 150, 403.

HERVA, E. and JOUPPILA, P. (1977). Mixed lymphocyte culture reactions between parental cells in pregnancy and puerperium. Acta Path. Microbiol. Scand. (C) 85, 99.

HILL, D.E., ARELLANO, C.P., IZUKAWA, T., HOLT, A.B. and CHEEK, D.B. (1970). Studies in infants and children with congenital rubella. Oxygen consumption, body water, cell mass, muscle and adipose tissue composition. John Hopkins Med. J. 128, 309.

HJELM, H., HJELM, K. and SJOQUIST, J. (1972). Protein A from Staphylococcus aureus. Its isolation by affinity chromatography and its use as an immunosorbent for isolation of immunoglobulins. FEBS lett. 28, 73.

HONEYMAN, M.C., DORMAN, D.C., MENSER, M.A., FORREST, J.M. GUINAN, J.J. and CLARK, P. (1975). HLA antigens in congenital rubella and the role of antigens 1 and 8 in the epidemiology of natural rubella. Tissue antigens 5, 12.

HOPKINS, J., McCONNELL, I. and LACHMANN, P.J. (1981). Specific selection of antigen-reactive lymphocytes into antigenically stimulated lymph nodes in sheep. J. Exp. Med. 153, 706.

HOSKINS, J.M. and PLOTKIN, S.A. (1967). Behaviour of rubella virus in human diploid cell strains. II. Studies of infected cells. Arch. ges. Virusforsch 21, 296.

HSU, C.C.S. (1974). Peripheral blood lymphocyte responses to phytohemagglutinin and pokeweed mitogen during pregnancy. Proc. Soc. Exp. Biol. Med. 146, 771.

HUBBERT, W.T., BRYNER, J.H., FERNELIUS, A.L., FRANK, G.H. and ESTES, P.C. (1973). Viral infection of the bovine fetus and its environment. Arch. ges. Virusforsch 41, 86.

HUCK, R.A. and ASTON, F.W. (1964). The "carrier" sow in swine fever. Vet. Rec. 76, 1151.

HUESNER, J. (1980). Three-dimensional visualisation of coated vesicle formation in fibroblasts. J. Cell Biol. 84, 560.

IIDA, T., TAJIMA, M. and MURATA, Y. (1973). Transmission of maternal antibodies to Sendai virus in mice and its significance in enzootic infection. J. Gen. Virol. 18, 247.

IMMUNISATION PRACTICES ADVISORY COMMITTEE (1981). Recommendation on rubella prevention. Cited by Orenstein and Greaves (1982).

ISAACS, A. and BARON, S. (1960). Antiviral action of interferon in embryonic cells. Lancet ii, 946.

JACKSON, A.D.M. and FISCH, L. (1958). Deafness following maternal rubella. Results of a prospective investigation. Lancet ii, 1241.

JARRETT, O. (1977). Prenatal viral infections and delayed postnatal disease. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p.515.

JEFFCOTT, L.B. (1974). Studies on passive immunity in the foal. J. Comp. Pathol. 84, 279.

JELLIFFE, D.B. (1976). World trends in infant feeding. Am. J. Clin. Nutr. 29, 1227.

JENKINS, D.M and HANCOCK, K.W. (1972). Maternal unresponsiveness to paternal histocompatability antigens in human pregnancy. Transplantation 13, 618.

JENKINSON, E.J. and BILLINGTON, W.D. (1974). Differential susceptibility of mouse trophoblast and embryonic tissue to immune cell lysis. Transplantation 18, 286.

JOHNSON, K.P. (1969). Mouse cytomegalovirus: placental infection. J. Infect. Dis. 120, 445.

JOHNSON, P.M. and BROWN, P.J. (1981). Fcγ receptors in the human placenta. Placenta 2, 355.

KALISS, N. and DAGG, M.K. (1964). Immune response engendered in mice by multiparity. Transplantation 2, 416.

KALTER, S.S., HELMKE, R.J., PANIGEL, M., HEBERLING, R.L. FELSBURG, P.J. and AXELROD, L.R. (1973). Observations of apparent C-type particles in baboon (Papio cynocephalus) placentas. Science 179, 1332.

KASAKURA, S. (1971). A factor in maternal plasma during pregnancy that suppresses the reactivity of mixed leukocyte cultures. J. Immunol. 107, 1296.

KENDRICK, J.W. (1973). Effects of the infectious bovine rhinotracheitis virus on the fetus. J. Am. Vet. Med. Assoc. 163, 852.

KENDRICK, J.W. and STRAUB, O.C. (1967). Infectious bovine rhinotracheitis - infectious pustular vulvovaginitis virus infection in pregnant cows. Am. J. Vet. Res. 28, 1269.

KENDRICK, J.W. and OSBURN, B.I. (1973). Immunologic response of the bovine fetus to inactivated infectious bovine rhinotracheitis - infectious pustular vulvovaginitis virus. Am. J. Vet. Res. 34, 1567.

KENDRICK, J.W., SCHNEIDER, L. and STRAUB, O.C. (1971). Placental reaction to the infectious bovine rhinotracheitis - infectious pustular vulvovaginitis virus. Am. J. Vet. Res. 32, 1045.

KHOURY, P.B., LLOYD, S.S., REID, W.A., WEINER, D.J., PHILLIPS, S.M. and SOULSBY, E.J.L. (1981). Kinetics and characterisation of antigen-binding and antibody-producing cells in the regional draining lymph nodes and spleen during initial murine schistosomiasis. Cell. Immunol. 59, 233.

KHUROO, M.S., TELI, M.R., SKIDMORE, S., SOFI, M.A. and KHUROO, M.I. (1981). Incidence and severity of viral hepatitis in pregnancy. Am. J. Med. 70, 252.

KILHAM, L., MARGOLIS, G. and COLBY, E.D. (1967). Congenital infections of cats and ferrets by feline panleukopenia virus manifested by cerebellar hypoplasia. Lab. Invest. 17, 465.

KIRBY, D.R.S., BILLINGTON, W.D. and JAMES, D.A. (1966). Transplantation of eggs to the kidney and uterus of immunised mice. Transplantation 4, 713.

KLEIN, J. (1978). H-2 mutations: their genetics and effect on immune functions. Adv. Immunol. 26, 55.

KNOX, A.W. (1950). Influence of pregnancy in mice on the course of infection with murine poliomyelitis virus. Proc. Soc. Exp. Biol. Med. 73, 520.

KONO, R., HIBI, M., HAYAKAWA, Y. and ISHII, K. (1969). Experimental vertical transmission of rubella virus in rabbits. Lancet i, 343.

KRUGMAN, S. and KATZ, S.L. (1974). Rubella immunisation: a five year progress report. N. Engl. J. Med. 290, 1375.

KURTZ, J.B., TOMLINSON, A.H. and PEARSON, J. (1982). Mumps virus isolated from a fetus. Brit. Med. J. i. 471.

LANCE, E.M., MEDAWAR, P.B. and TAUB, R.N. (1973). Antilymphocyte serum. Adv. Immunol. 17, 1.

LANCET (1973). Rubella reinfection and the fetus. Lancet i, 978.

LANCET (1974). Congenital cytomegalovirus infection - more problems. Lancet i, 845.

LANG, D.J., KUMMER, J.F. and HARTLEY, D.P. (1974). Cytomegalovirus in semen. Persistence and demonstration in extracellular fluids. N. Engl. J. Med. 291, 121.

LANSDOWN, A.B.G. (1975). Pathological changes in pregnant mice infected with coxsackievirus B3 and given dietary casein hydrolysate supplement. Br. J. Exp. Pathol. 56, 373.

LANSDOWN, A.B.G. and COID, C.R. (1974). Pathological changes in pregnant mice infected with coxsackie B3 virus as a possible cause of retarded foetal development. Br. J. Exp. Pathol. 55, 101.

LARSON, H.E., PARKMAN, P.D., DAVIS, W.J., HOPPS, H.E. and MEYER, H.M. (1971). Inadvertent rubella virus vaccination during pregnancy. N. Engl. J. Med. 284, 870.

LECK, I. (1963). Incidence of malformations following influenza epidemics. Br. J. Prev. Soc. Med. 17, 70.

LECK, I. and STEWARD, J.K. (1972). Incidence of neoplasms in children born after influenza epidemics. Br. Med. J. iv, 631.

LEDBETTER, J.A., ROUSE, R.V., MICKLEM, H.S. and HERZENBERG, L.A. (1980). T cell subsets defined by expression of Ly-1, 2, 3 and Thy-1 antigens. J. Exp. Med. 152, 280.

LEIKIN, S. (1972). Depressed maternal lymphocyte response to phytohaemagglutinin in pregnancy. Lancet ii, 43.

LEVEY, R.H. and MEDAWAR, P.B. (1966). Nature and mode of action of antilymphocytic serum. Proc. Natl. Acad. Sci. U.S.A. 56, 1130.

LEVY, J.A. (1973). Xenotropic viruses: murine leukemia viruses associated with NIH Swiss, NZB, and other mouse strains. Science 182, 1151.

LINDH, E. (1975). Increased resistance of immunoglobulin A dimers to proteolytic degradation after binding of secretory component. J. Immunol. 114, 284.

LOGAN, W.P.D. (1951). Incidence of congenital malformations and their relation to virus infections during pregnancy. Brit. Med. J, ii, 641.

LOWRIE, D.B., TOMS, G.L. and PEARCE, J.H. (1977). Mechanisms of microbial pathogenicity during pregnancy in relation to maternal and fetal health. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. 207.

MACKANESS, G. (1978). Delayed hypersensitivity. In "Immunological diseases". 3rd Edn. Vol.1. Ed. M. Samter. Little, Brown and Co. p.281.

MAKINODAN, T. and PETERSON, W.J.(1962). Relative antibody-forming capacity of spleen cells as a function of age. Proc. Natl. Acad. Sci. USA 48, 234.

MARONI, E.S. and DE SOUSA, M.A.B. (1973). The lymphoid organs during pregnancy in the mouse. A comparison between a syngeneic and an allogeneic mating. Clin. Exp. Immunol. 13, 107.

MASSON, P.L., DELIRE, M. and CAMBIASO, C.L. (1977). Circulating immune complexes in normal human pregnancy. Nature 266, 542.

MATA, L., URRUTIA, J.J., SERRATO, G., MOHS, E. and CHIN, T.D.Y. (1977). Viral infections during pregnancy and in early life. Am. J. Clin. Nutr. 30, 1834.

MATHUR, A., ARORA, K.L. and CHATURVEDI, U.C. (1981). Congenital infection of mice with Japanese encephalitis virus. Infect. Immun. 34, 26.

MATRE, R. and HAUGEN, A. (1978). The placental Fcγ receptors studied using immune complexes of peroxidase. Scand. J. Immunol. 8, 187.

McCANCE, D.J. and MIMS, C.A. (1977). Transplacental transmission of polyoma virus in mice. Infect. Immun. 18, 196.

McFARLAND, H.F. (1974). In vitro studies of cell-mediated immunity in an acute viral infection. J. Immunol. 113, 173.

MEADE, T.W. and ATKINSON, A.B. (1977). Economic consequences of infection in human pregnancy. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. 551.

MEDAWAR, P.B. and SPARROW, E.M. (1956). The effects of andrenocortical hormones, adrenocorticotrophic hormone and pregnancy on skin transplantation immunity in mice. J. Endrocrinol. 14, 240.

MEDEARIS, D.N. (1982). CMV immunity: imperfect but protective. N. Engl. J. Med. 306, 985.

MELLANDER, L., CARLSSON, B., DAHLGREN, U. and HANSON, L.A. (1981). Humoral and cellular immunities transmitted by breastmilk. In "Immunological aspects of infection in the fetus and newborn". Eds. H.P. Lambert and C.B.S. Wood. Academic Press. p. 139.

MENSER, M.A. and FORREST, J.M. (1974). Rubella - high incidence of defects in children considered normal at birth. Med. J. Aust. 1, 123.

MENSER, M.A. and FORREST, J.M.S. (1975). Rubella vaccination and pregnancy. Med. J. Aust. 1, 794.

MILLER, J.F.A.P. (1966). Immunity in the fetus and the newborn. Br. Med. Bull. 22, 21.

MIMS, C.A. (1968). Pathogenesis of viral infections of the fetus. Prog. Med. Virol. 10, 194.

MIMS, C.A. (1969). Effect on the fetus of maternal infection with lymphocytic choriomeningitis (LCM) virus . J. Infect. Dis. 120, 582.

MIMS, C.A. (1976a). In "The pathogenesis of infectious disease". Academic Press.

MIMS, C. (1976b). Comparative aspects of infective malformations. Br. Med. Bull. 32, 84.

MIMS, C.A. (1981). Vertical transmission of viruses. Microbiol. Rev. 45, 267.

MODLIN, J.F., HERRMANN, K., BRANDLING-BENNETT, A.D., EDDINS, D.L. and HAYDEN, G.F. (1976). Risk of congenital abnormality after inadvertent rubella vaccination of pregnant women. N. Engl. J. Med. 294, 972.

MOHR, J.A. (1973). The possible induction and/or acquisition of cellular hypersensitivity associated with ingestion of colostrum. J. Pediatr. 82, 1062.

MONTGOMERY, R., YOUNGBLOOD, L. and MEDEARIS, D.N. (1972). Recovery of cytomegalovirus from the cervix in pregnancy. Pediatrics 49, 524.

MORGAN, W.J.B. and WRATHALL, A.E. (1977). Aetiology, diagnosis, prevention and control of infections affecting pregnancy in farm animals. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. 53.

MULLBACHER, A. (1981). Neonatal tolerance to allo-antigens alters major histocompatability complex-restricted response patterns. Proc. Natl. Acad. Sci. U.S.A. 78, 7689.

MURASKO, D.M. and BLANK, K.J. (1980). Transplacental interferon-mediated effect in AKR mice. Virology 106, 148.

MUSCOPLAT, C.C., THOEN, C.O., CHEN, A.W. and JOHNSON, D.W. (1974). Macrophage-dependent lymphocyte immuno-stimulation in cattle infected with Mycobacterium bovis and with Mycobacterium avium. Infect. Immun. 10, 675.

NAEYE, R.L. and BLANC, W. (1965). Pathogenesis of congenital rubella. J. Am. Med. Assoc. 194, 1277.

NAHMIAS, A.J., JOSEY, W.E., NAIB, Z.M., FREEMAN, M.G., FERNANDEZ, R.J. and WHEELER, J.H. (1971). Perinatal risk associated with maternal genital herpes simplex virus infection. Am. J. Obstet. Gynecol. 110, 825.

NAHMIAS, A.J., VISINTINE, A.M., REIMER, C.B., BUONO, I.D., SHORE, S.L. and STARR, S.E. (1975). Herpes simplex virus infection of the fetus and newborn. Prog. Clin. Biol. Res. 3, 63.

NASH, D.R. and HOLLE, B. (1973). Local and systemic cellular immune responses in guinea pigs given antigen parenterally or directly into the lower respiratory tract. Clin. Exp. Immunol. 13, 573.

NEJAMKIS, M.R., NOTA, N.R., WEISSENBACHER, M.C., DE GUERRERO, L.B. and GIOVANNIELLO, O.A. (1975). Passive immunity against junin virus in mice. Acta Virol. (Praha) 19, 237.

NICKLIN, S. and BILLINGTON, W.D. (1979). Macrophage activity in mouse pregnancy. J. Reprod. Immunol. 1, 117.

NOSSAL, G.J.V., ABBOT, A., MITCHELL, J. and LUMMUS, Z. (1968). Antigens in immunity. XV Ultrastructural features of antigen capture in primary and secondary lymphoid follicles. J. Exp. Med. 127, 277.

OCKLEFORD, C.D., and WHYTE, A. (1977). Differentiated regions of human placental cell surface associated with exchange of materials between maternal and foetal blood: coated vesicles. J. Cell. Sci. 25, 293.

OGRA, S.S., and OGRA, P.L. (1978). Immunologic aspects of human colostrum and milk. J. Pediatr. 92, 550.

OGRA, P.L., KERR-GRANT, D., UMANA, G., DZIERBA, J. and WEINTRAUB, D. (1971). Antibody response in serum and nasopharynx after naturally acquired and vaccine-induced infection with rubella virus. N. Engl. J. Med. 285, 1333.

OGRA, S.S., WEINTRAUB, D. and OGRA, P.L. (1977). Immunologic aspects of human colostrum and milk. J. Immunol. 119, 245.

OGRA, P.L., FISHAUT, M. and THEODORE, C. (1980). Immunology of breast milk: maternal neonatal interactions. In "Human milk. Its biological and social value". Eds. S. Freier and A.I. Eidelman. Excerpta Medica. p. 115.

ORENSTEIN, W.A. and GREAVES, W.L. (1982). Congenital rubella syndrome: a continuing problem. J. Am. Med. Assoc. 247, 1174.

OSBURN, B.I., SILVERSTEIN, A.M., PRENDERGAST, R.A., JOHNSON, R.T. and PARSHALL, C.J. (1971a). Experimental viral-induced congenital encephalopathies. I. Pathology of hydranencephaly and porencephaly caused by bluetongue vaccine virus. Lab. Invest. 25, 197.

OSBURN, B.I., JOHNSON, R.T, SILVERSTEIN, A.M., PRENDERGAST, R.A., JOCHIM, M.M. and LEVY, S.E. (1971b). Experimental viral-induced congenital encephalopathies. II. The pathogenesis of bluetongue vaccine virus infection in fetal lambs. Lab. Invest. 25, 206.

PARSONSON, I.M., DELLA-PORTA, A.J. and SNOWDON, W.A. (1977). Congenital abnormalities in newborn lambs after infection of pregnant sheep with Akabane virus. Infect. Immun. 15, 254.

PARSONSON, I.M., O'HALLORAN, M.L., ZEE, Y.C. and SNOWDON, W.A. (1979). The effects of bovine viral diarrhoea-mucosal disease (BVD) virus on the ovine foetus. Vet. Microbiol. 4, 279.

PARSONSON, I.M., DELLA-PORTA, A.J. and SNOWDON, W.A. (1981). Developmental disorders of the fetus in some arthropod-borne virus infections. Am. J. Trop. Med. Hyg. 30, 660.

PAVIA, C.S., and STITES, D.P. (1979). Humoral and cellular regulation of alloimmunity in pregnancy. J. Immunol. 123, 2194.

PECKHAM, C.S. (1974). Clinical and serological assessment of children exposed in utero to confirmed maternal rubella. Br. Med. J. i, 259.

PENCE, H., PETTY, W.M. and ROCKLIN, R.E. (1975). Suppression of maternal responsiveness to paternal antigens by maternal plasma. J. Immunol. 114, 525.

PIJNENBORG, R., ROBERTSON, W.B., BROSENS, I. and DIXON, G. (1981). Trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. Placenta 2, 71.

PITT, J. (1979). The milk mononuclear phagocyte. Pediatrics 64, 745.

PITTARD, W.B., POLMAR, S.H. and FANAROFF, A.A. (1977). The breastmilk macrophage: a potential vehicle for immunoglobulin transport. J. Reticuloendothel. Soc. 22, 597.

PLATEAU, E., VANNIER, P., and TILLON, J.P. (1980). Atypical hog cholera infection: viral isolation and clinical study of in utero transmission. Am. J. Vet. Res. 41, 2012.

PLOTKIN, S.A. (1975). Routes of fetal infection and mechanisms of fetal damage. Am. J. Dis. Child. 129, 444.

PLOTKIN, S.A. and VAHERI, A. (1967). Human fibroblasts infected with rubella virus produce a growth inhibitor. Science 156, 659.

PLOTKIN, S.A., BOUÉ, A. and BOUE, J.G. (1965). The in vitro growth of rubella virus in human embryo cells. Am. J. Epidemiol. 81, 71.

PLUM, J., THIERY, M. and SABBE, L. (1978). Distribution of mononuclear cells during pregnancy. Clin. Exp. Immunol. 31, 45.

PORTER, P. (1969). Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorbtion by the neonatal piglet. Biochim. Biophys. Acta. 181, 381.

PORTER, P. (1976). Intestinal absorption of colostral IgA anti-E. coli antibodies by the neonatal piglet and calf. In "Maternofoetal transmission of immunoglobulins". Ed. W.A. Hemmings. Cambridge University Press. p. 397.

PORTER, D.D., LARSEN, A.E., and PORTER, H.G. (1977). Reduced severity of lesions in mink infected transplacentally with aleutian disease virus. J. Immunol. 119, 872.

POTTER, J.E., BANATVALA, J.E. and BEST, J.M. (1973). Interferon studies with Japanese and U.S. rubella virus strains. Br. Med. J. i, 197.

PREBLUD, S.R., NIEBURG, P.I. and HINMAN, A.R. (1978). Rubella vaccination and pregnancy. Br . Med. J. ii, 960.

RAGHUPATHY, R., SINGH, B., LEIGH, J.B. and WEGMANN, T.G. (1981). The ontogeny and turnover kinetics of paternal H-2K antigenic determinants on the allogeneic murine placenta. J. Immunol. 127, 2074.

RAO, A.R. (1972). In "Smallpox". The Kothari Book Depot, Bombay. p. 120.

RAWLS, W.E. and MELNICK, J.L. (1966). Rubella virus carrier cultures derived from congenitally infected infants. J. Exp. Med. 123, 795.

REGISTRAR GENERAL (1972-3). Statistical review of England and Wales. Supplement on abortion. Cited by Tobin et al. (1977).

REITER, B. (1981). The contribution of milk to resistance to intestinal infection in the newborn. In "Immunological aspects of infection in the fetus and newborn". Eds. H.P. Lambert and C.B.S. Wood. Academic Press. p. 155.

REVILLARD, J.P., ROBERT, M., BETUEL, H., LATOUR, M., BONNEAU, M., BROCHIER, J. and TRAEGER, J. (1972). Inhibition of the mixed lymphocyte reaction by antibodies. Transplant. Proc. 4, 173.

REYNOLDS, D.W., STAGNO, S., STUBBS, K.G., DAHLE, A.J., LIVINGSTON, M.M., SAXON, S.S., ALFORD, C.A. (1974). Inapparent congenital cytomogalovirus infection with elevated cord IgM levels. Causal relation with auditory and mental deficiency. N. Engl. J. Med. 290, 291.

ROBERT, M., BETUEL, H. and REVILLARD, J.P. (1973). Inhibition of the mixed lymphocyte reaction by sera from multipara. Tissue Antigens 3, 39.

ROCKLIN, R.E., KITZMILLER, J.L., CARPENTER, C.B., GAROVOY, M.R. and DAVID, J.R. (1976). Maternal-fetal relation. Absence of an immunological blocking factor from the serum of women with chronic abortions. N. Engl. J. Med. 295, 1209.

RODEWALD, R. (1980). Distribution of immunoglobulin G receptors in the small intestine of the young rat. J. Cell. Biol. 85, 18.

ROITT, I.M. (1974). In "Essential Immunology". 2nd Edition. Blackwell Scientific Publications. p.137.

ROUX, M.E., McWILLIAMS, M., PHILLIPS-QUAGLIATA, J.M., WEISZ-CARRINGTON, P. and LAMM, M.E. (1977). Origin of IgA-secreting plasma cells in the mammary gland. J. Exp. Med. 146, 1311.

RUGH, R. (1968). In "The mouse. It's reproduction and development". Burgess Publishing Co.

RYLATT, D.B. and PARISH, C.R. (1982). Protein determination on an automatic spectrophotometer. Anal. Biochem. 121, 213.

ST. HILL, C.A., FINN, R. and DENYE, V. (1973). Depression of cellular immunity in pregnancy due to a serum factor. Br. Med. J. iii, 513.

SCHECHTER, B. (1980). In "Lymphocyte stimulation". Ed. A. Castellani. Plenum Press. p. 1.

SCHLESINGER, J.J. and COVELLI, H.D. (1977). Evidence for transmission of lymphocyte responses to tuberculin by breast feeding. Lancet ii, 529.

SEARLE, R.F., JENKINSON, E.J. and JOHNSON, M.H. (1975). Immunogenicity of mouse trophoblast and embryonic sac. Nature 255, 719.

SENELAR, R. and BUREAU, J.P. (1979). Inhibitory effect of pregnancy on the migration of the inflammatory cells: a quantitative histological study. Br. J. Exp. Pathol. 60, 286.

SEPPALA, M. and VAHERI, A. (1974). Natural rubella infection of the female genital tract. Lancet i, 46.

SEPPALA, I., SARVAS, H., PETERFY, F. and MAKELA, O. (1981). The four subclasses of IgG can be isolated from mouse serum using protein A-sepharose. Scand. J. Immunol. 14, 335.

SEVER, J.L. (1962). Application of a microtechnique to viral serological investigations. J. Immunol. 88, 320.

SEVER, J. and WHITE, L.R. (1968). Intrauterine viral infections. Annu. Rev. Med. 19, 471.

SHAW, A.R.E., DASGUPTA, M.K., KOVITHAVONGS, T., JOHNY, K.V., LE RICHE, J.C., DOSSETOR, J.B. and McPHERSON, T.A. (1979). Humoral and cellular immunity to paternal antigens in trophoblastic neoplasia. Int. J. Cancer 24, 586.

SIEGEL, M. and GREENBERG, M. (1955). Incidence of poliomyelitis in pregnancy. Its relation to maternal age, parity and gestational period. N. Engl. J. Med. 253, 841.

SIEGEL, M. and GREENBERG, M. (1960). Fetal death, malformation and prematurity after maternal rubella. Results of a prospective study 1949-1958. N. Engl. J. Med. 262, 389.

SIEGEL, M and FUERST, H.T. (1966). Low birth weight and maternal virus diseases. J. Am. Med. Assoc. 197, 680.

SIEGEL, M., FUERST, H.T. and PERESS, N.S. (1966). Comparative fetal mortality in maternal virus diseases. A prospective study on rubella, measles, mumps, chicken pox and hepatitis. N. Engl. J. Med. 274, 768.

SIEGLER, H.F. and METZGAR, R.S. (1970). Embryonic development of human transplantation antigens. Transplantation 9, 478.

SIITERI, P.K. and STITES, D.P. (1982). Immunologic and endocrine interrelationships in pregnancy. Biol. Reprod. 26, 1.

SIITERI, P.K., FEBRES, F., CLEMENS, L.E., CHANG, R.J., GONDOS, B. and STITES, D.P. (1977). Progesterone and maintenance of pregnancy: is progesterone nature's immunosuppressant? Ann. N.Y. Acad. Sci. 286, 384.

SILVERSTEIN, A.M. (1972). Immunological maturation in the foetus: modulation of the pathogenesis of congenital infectious diseases. In "Ontogeny of acquired immunity". Ciba Foundation Symposium. Elsevier, Excerpta Medica, North-Holland. p. 16.

SILVERSTEIN, A.M., UHR, J.W., KRANER, K.L. and LUKES, R.J. (1963). Fetal response to antigenic stimulus. II. Antibody production by the fetal lamb. J. Exp. Med. 117, 799.

SIMMONS, R.L., LIPSCHULTZ, M.L., RIOS, A. and RAY, P.K. (1971). Failure of neuraminidase to unmask histocompatability antigens on trophoblast. Nature (New Biol.) 231, 111.

SISSONS, J.G.P. and OLDSTONE, M.B.A. (1980). Antibody mediated destruction of virus infected cells. Adv. Immunol. 29, 209.

SKINNER, H.H. and KNIGHT, E.H. (1971). Monitoring mouse stocks for lymphocytic choriomeningitis virus - a human pathogen. Lab. Anim. 5, 73.

SMITH, R.N. and POWELL, A.E. (1977). The adoptive transfer of pregnancy-induced unresponsiveness to male skin grafts with thymus-dependent cells. J. Exp. Med. 146, 899.

SNOW, C. and HILGARD, H.R. (1981). Local cellular immunity in the draining lymph nodes of mice after immunisation with histocompatability alloantigens. Transplantation 32, 29.

SOHNLE, P.G. and COLLINS-LECH, C. (1981). Lymphocyte transformation and the number of antigen-responsive cells in humans. J. Immunol. 127, 612.

SOOTHILL, J.F., HAYES, K. and DUDGEON, J.A. (1966). The immunoglobulins in congenital rubella. Lancet i, 1385.

STAGNO, S., REYNOLDS, D.W., LAKEMAN, A., CHARAMELLA, L.J. and ALFORD, C.A. (1973). Congenital cytomegalovirus infection: consecutive occurrence due to viruses with similar antigenic compositions. Pediatrics 52, 788.

STAGNO, S., REYNOLDS, D.W., TSIANTOS, A., FUCCILLO, D.A., LONG, W. and ALFORD, C.A. (1975). Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natally acquired cytomegalovirus infections. J. Infect. Dis. 132, 568.

STAGNO, S., PASS, R.F., DWORSKY, M.E., HENDERSON, R.E., MOORE, E.G., WALTON, P.D. and ALFORD, C.A. (1982). Congenital cytomegalovirus infection. N. Engl. J. Med. 306, 945.

STERN, H. and TUCKER, S.M. (1973). Prospective study of cytomegalovirus infection in pregnancy. Br. Med. J. ii, 268.

STIEHM, E.R., AMMANN, A.J. and CHERRY, J.D. (1966). Elevated cord macroglobulins in the diagnosis of intrauterine infection. N. Engl. J. Med. 275, 971.

STIMSON, W.H., HUNTER, I.C. and MANOS, C. (1977). Comparison of the effects of human pregnancy serum and anti-inflammatory compounds on the release of lysosomal enzymes from macrophages. Br. J. Exp. Pathol. 58, 434.

STIMSON, W,H., STRACHAN, A.F. and SHEPHERD, A. (1979). Studies on the maternal immune response to placental antigens - absence of a blocking factor from the blood of abortion prone women. Br. J. Obstet. Gynaecol. 86, 41.

STITES, D.P., PAVIA, C.S., CLEMENS, L.E., KUHN, R.W. and SIITERI, P.K. (1979). Immunologic regulation in pregnancy. Arthritis Rheum. 22, 1300.

STOTT, J.L., LAUERMAN, L.H. and LUEDKE, A.J. (1982). Bluetongue virus in pregnant elk and their calves. Am. J. Vet. Res. 43, 423.

SUNDERLAND, C.A., REDMAN, C.W.G. and STIRRAT, G.M. (1981). HLA A, B, C antigens are expressed on nonvillous trophoblast of the early human placenta. J. Immunol. 127, 2614.

SUZUKI, K. and TOMASI, T.B. (1979). Immune responses during pregnancy. Evidence of suppressor cells for splenic antibody response. J. Exp. Med. 150, 898.

TACHIBANA, D.K. and ROSENBERG, L.T. (1966). Fetal synthesis of Hc', a component of mouse complement. J. Immunol. 97, 213.

TAI, C. and HALASZ, N.A. (1967). Histocompatability antigen transfer in utero: tolerance in progeny and sensitisation in mother. Science 158, 125.

TAUB, R.N. (1969). Lymphocyte kinetics and lymphoid tissue morphology accompanying immunosuppression by antilymphocyte serum (ALS). Antibiot. Chemother. 15, 250.

TAYLOR-ROBINSON, D. (1977). Infections and infertility in man and animals. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. 141.

TAYLOR, P.V. and HANCOCK, K.W. (1975). Antigenicity of trophoblast and possible antigen-masking effects during pregnancy. Immunology 28, 973.

TIKASINGH, E.S., SPENCE, L. and DOWNS, W.G. (1966). The use of adjuvant and sarcoma 180 cells in the production of mouse hyperimmune ascitic fluids to arboviruses. Am. J. Trop. Med. Hyg. 15, 219.

TOBIN, J.O'H., JONES, D.M. and FLECK, D.G. (1977). Aetiology, diagnosis, prevention and control of infections affecting pregnancy in humans. In "Infections and pregnancy". Ed. C.R. Coid. Academic Press. p. 1.

TOMASI, T.B., TAN, E.M., SOLOMON, A. and PRENDERGAST, R.A. (1965). Characteristics of an immune system common to certain external secretions. J. Exp. Med. 121, 101.

TONDURY, G. and SMITH, D.W. (1966). Fetal rubella pathology. J. Pediatr. 68, 867.

TSE, H.Y., SCHWARTZ, R.H. and PAUL, W.E. (1980). Cell-cell interactions in the T cell proliferative response. I. Analysis of the cell types involved and evidence for nonspecific T cell recruitment. J. Immunol. 125, 491.

UHR, J.W., DANCIS, J. and FINKELSTEIN, M.S. (1963). Passage of bacteriophage ØX 174 across the placenta in guinea pigs. Proc. Soc. Exp. Biol. Med. 113, 391.

VAHERI, A., VESIKARI, T., OKER-BLOM, N., SEPPALA, M., PARKMAN, P.D., VERONELLI, J. and ROBBINS, F.C. (1972). Isolation of attenuated rubella-vaccine virus from human products of conception and uterine cervix. N. Engl. J. Med. 286, 1071.

van der MEULEN, J.A., McNABB, T.C., HAEFFNER-CAVAILLON, N., KLEIN, M. and DORRINGTON, K.J. (1980). The Fcγ receptor on human placental plasma membrane. I. Studies on the binding of homologous and heterologous immunoglobulin G. J. Immunol. 124, 500.

VAN ZON, A.A.J.C. and ELING, W.M.C. (1980). Depressed malarial immunity in pregnant mice. Infect. Immun. 28, 630.

WALKER, J.S., FREEMAN, C.B. and HARRIS, R. (1972). Lymphocyte reactivity in pregnancy. Br. Med. J. iii, 469.

WEGMANN, T.G. (1981). The presence of Class I MHC antigens at the maternal-fetal interface and hypotheses concerning the survival of the murine fetal allograft. J. Reprod. Immunol. 3, 267.

WEIL, M.L., ITABASHI, H.H., CREMER, N.E., OSHIRO, L.S., LENNETTE, E.H. and CARNAY, L. (1975). Chronic progressive panencephalitis due to rubella virus simulating subacute sclerosing panencephalitis. N. Engl. J. Med. 292, 994.

WEINSTEIN, L., AYCOCK, W.L. and FEEMSTER, R.F. (1951). The relation of sex, pregnancy and menstruation to susceptibility in poliomyelitis. N. Engl. J. Med. 245, 54.

WELLER, T.H. (1971). The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. N. Engl. J. Med. 285, 203.

WILD, A.E. (1975). Role of the cell surface in selection during transport of proteins from mother to foetus and newly born. Philos. Trans. R. Soc. Lond. Ser.B. 271, 395.

WILD, A.E. (1981). Distribution of Fcγ receptors on isolated gut enterocytes of the suckling rat. J. Reprod. Immunol. 3, 283.

WILD, A.E. and RICHARDSON, L.J. (1979). Direct evidence for pH-dependent Fc receptors on proximal enterocytes of the suckling rat gut. Experientia 35, 838.

WINKLER, C.E., GIBBONS, D.F. and SHAW, I.G. (1975). Observations on the experimental transmissability of border disease in sheep. Br. Vet. J. 131, 32.

WOODRUFF, J.F. and WOODRUFF, J.J. (1975). T lymphocyte interaction with viruses and virus-infected tissues. Prog. Med. Virol. 19, 120.

WYNN, R.M. (1969). Noncellular components of the placenta. Am. J. Obstet. Gynecol. 103, 723.

YAMASHITA, K., WAKE, N., ARAKI, T., ICHINOE, K. and MAKOTO, K. (1979). Human lymphocyte antigen expression in hydatiform mole: androgenesis following fertilisation by a haploid sperm. Am. J. Obstet. Gynecol. 135, 597.

YOUTANANUKORN, V. and MATANGKASOMBUT, P. (1973). Specific plasma factors blocking human maternal cell mediated immune reaction to placental antigens. Nature (New Biol.) 242, 110.

YOUTANANUKORN, V., MATANGKASOMBUT, P. and OSATHANONDH, V. (1974). Onset of human maternal cell-mediated immune reaction to placental antigens during the first pregnancy. Clin. Exp. Immunol. 16, 593.

YU, V.Y.H., WALLER, C.A., MACLENNAN, I.C.M. and BAUM, J.D. (1975). Lymphocyte reactivity in pregnant women and newborn infants. Br. Med. J. i, 428.

ZEALLY, H. and EDMOND, E. (1982). Rubella screening and immunisation of schoolgirls: results six to seven years after vaccination. Br. Med. J. i, 382.

ZINKERNAGEL, R.M. and ALTHAGE, A. (1977). Antiviral protection by virus-immune cytotoxic T cells: infected target cells are lysed before infectious virus progeny is assembled. J. Exp. Med. 145, 644.

ZINKERNAGEL, R.M. and DOHERTY, P.C. (1979). MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T cell restriction-specificity, function and responsiveness. Adv. Immunol. 27, 51.